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HERMIONE

NOVEL ANTICANCER THERAPEUTICS BASED ON MODULATION OF APOPTOSIS THROUGH DEPENDENCE RECEPTORS

FP6 - Specific Targeted Research Projects (STREP)

Priority T1 – Life Sciences

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1 Summary of project results

The initial goals of the HERMIONE project were first to provide more knowledge on a recent concept called the dependence receptor (DR) notion. CLB Partner recently described that some receptors are not inactive in the absence of ligand but instead trigger apoptosis and thus render cell to be dependent for their survival on the presence of the ligand in the close environment. It was proposed that this receptors are tumor suppressors and as such represent a safeguard mechanism to limit tumor progression. It was then proposed that a large fraction of breast cancer, one of the ligand of these DRs, netrin-1 is upregulated and provides a survival advantage for tumor development. The second goal of the project was thus to propose a therapeutic strategy aiming at interfering with DRs ligands. The DRs analysed during the course of this HERMIONE projects were the netrin-1 receptors DCC and UNC5H1-2-3, the GDNF receptor RET, the NT-3 receptor TrkC and the putative dependence receptor KAI1.

The first achievements of HERMIONE were then to get more insights into the molecular mechanisms triggered by these unbound receptors. Achievements included the work by UNIL on the adaptor protein PIDD. UNIL indeed obtained the structure of the PIDDosome and showed that PIDD is submitted to autoproteolysis. UNIBO was able to provide a new link between RET and cell death by showing using the split-ubiquitin assay the implication of AIP (Aryl Hydrocarbon receptor Interacting Protein) in the cell death induced by RET and mapped the interaction to the pro-apoptotic domain of RET. CLB also discovered by a siRNA screen the importance of the phosphatase PP2A or more exactly of its structural subunit PR65¤ (PPP2R1B) as a key regulator of UNC5H2 pro-apoptotic activity.

An important achievement of IMP was to demonstrate KAI1 as a new dependence receptor. IMP knew that KAI1 expression was associated with cell death but to fulfil the requirement for being a dependence receptor it was important to define a ligand for this orphan receptor that could block KAI1-induce apoptosis. IMP was thus able to identify a putative ligand for KAI1 that blocks KAI1-induced cell death. Thus, this lead to the demonstration that KAI1 is a dependence receptor and that this ligand may turn as a target for cancer.

The second main achievement of the HERMIONE project was to provide the proof-of concept that targeting ligand/DRs interaction is a promising alternative therapeutic strategy. Most of this proof of concept was made on netrin-1 and its receptors, even tough some data were reported on NT-3/TrkC. APOTECH/UNIBO/CLB in particular showed that netrin-1 is up-regulated in a large fraction of cancer types. Crucial tools like netrin-1 and UNC5H antibodies were produced by APOTECH and distributed to CLB and UNIBO to screen for netrin-1/receptors expression in cancer. Moreover, strong *in vivo* results were obtained and published. They provided the proof of concept that targeting netrin-1 in different animal models inhibits tumor development or induces tumor regression, supporting the idea that interfering with netrin-1/receptors can represent an alternative therapeutic approach in different pathologies, like metastatic breast cancer, lung cancers, neuroblastoma and colorectal cancers.

As a first approach to provide candidate drugs that could act as interferents for netrin-1/receptors, CEA and CLB developed a robust ELISA test that has been transferred to the CEA robots. Then, a first "small" screen has been performed on 1200 compounds and has allowed the identification of hits, that will be tested out of the scope of the HERMIONE projects.

To ensure a better communication between the partners and towards the public and the scientific community, a logo was created, a public web site (http://www.hermione-project.com) was designed and an intranet web platform was developed (https://www.myndsphere.com). These activities were coordinated by ALMA, with the contribution of its subcontractor and of all the other participants.

Thus the HERMIONE project fulfilled most of these initial goals providing a strong link between basic research to translational research toward identification of candidate drugs to fight cancer.

2 Contractors involved

The HERMIONE consortium included high level research centres, with complementary expertise in cell biology (IMP, CLB, UNIL), genetics (UNIBO, CLB), animal models (CEA), or drug development (CEA).

A consulting company, **ALMA**, was dedicated to the management of the project.

Full names of **partners** are given below:

Centre Léon Bérard - CLB (FR)
University of Lausanne - UNIL (CH)
University of Bologna - UNIBO (IT)
APOTECH Corporation - APO (CH)
Imperial College London - IMP (UK)
Laboratoires SERVIER - SERVIER (FR)
ALMA Consulting Group - ALMA (FR)
Commissariat à l'Energie Atomique - CEA (FR)

3 Scientific approach

To achieve its ambitious objectives, apart the management tasks, the HERMIONE work programme has been split down into 5 work packages:

- 1 workpackage (WP1) was dedicated to the integration of DR into extra- and intra-cellular signal transduction pathways
- 2 workpackages (WP 2 and 3) were dedicated to the validation, *in vitro* and *in vivo* of the importance of the dependence receptors in tumorigenesis.
- 1 workpackage (WP 4) was dedicated to the demonstration that dependence receptors and their ligand could be the basis of a relevant and original therapeutic strategy.
- 1 workpackage (WP6) was dedicated to the **management of knowledge** (intellectual property rights, dissemination activities and exploitation plan).

The objectives of main technical WPs are detailed below:

WP1 **Signalling by Dependence Receptors**

1. To identify new molecules that link DRs to the pro-apoptotic pathway.
   - Identify the ligand of KAI1
   - Identify new interactors of DRs
   - Determine whether Caspase-2 is involved in the pro-apoptotic pathway downstream of DRs
   - Determine whether DRs mediate apoptosis through regulation of transcription
2. To generate antibodies against novel components of the pro-apoptotic pathway for the validation of targets of potential therapeutic interest.

WP2 **Dependence receptors and their ligands in human tumours**

1. To validate the importance of DRs in human tumours and cell lines and generate new markers for diagnosis and prognosis.
   - Generation of antibodies
   - Identification of autocrine expression of DR ligands
   - Generation of ELISA detection kits for netrin-1/ KAI1 ligand
   - Search for mutations and LOH for DRs and new transducers
   - Evaluation of DR ligand or new transducers as diagnosis or prognosis markers.

WP3 **Murine models of tumoral development**

To provide *in vivo* validation of the variations of expression of DRs and their ligands and effect on tumorigenesis

1. Generate transgenic murine models
2. Analyse the effect of inhibiting DRs-apoptotic function on tumour development using models obtained in 1. and alternative existing models

WP4 **Dependence receptors as therapeutic targets**

1. Pre-clinical validation of soluble Netrin-1 receptors as anti-cancer therapy
   - ✓ Block netrin-1 with a recombinant Fc fragment and validate that it interferes with tumour progression in metastatic breast cancer and colon in mice.
   - ✓ Binding assay
   - ✓ Functional cell-based assay.
2. New targets: KAI1 ligand and intracellular components of the apoptotic pathway.
   - ✓ Block KAI1 ligand with a recombinant Fc fragment to validate that it interferes with tumour progression in prostate cancer in mice.
   - ✓ Screen for inhibitors of intracellular components of the pro-apoptotic pathway

4 Work performed and achievements

WP1 – Signalling by Dependence Receptors

1 State-of-the art at project start

Dependence receptors appear to constitute a selective advantage for a tumour cell when they loose their death activity by mutation, LOH or by the gain of autocrine expression of the ligand. Therefore, dependence receptors appear to be original targets for combating cancer, far from the classical highways of research on apoptosis-associated targets (like p53, Bcl-2 family members, Inhibitors of apoptosis or PI3/MAPK kinases) and potential candidates to be studied in the aim of developing novel cancer therapeutics. In order to achieve this, proper understanding of the way these receptors induce apoptosis, i.e. deciphering the signalling pathways acting downstream of DRs is of great importance. Along this line, the discovery of new dependence receptors is crucial to extend the number of potential targets. When we started this project we realised that the KAI1 (identified by IMP in a screen for apoptosis-inducing genes) receptor displayed all the traits of a DR although its ligand had not yet been described when we filed the application to the EU: (i) KAI1 is a metastasis suppressor and is down-regulated in any metastasis forming tumours and can inhibit this process when re-constitute, (ii) forced expression of KAI1 induces apoptosis in the absence of any extracellular ligand, (iii) the intracellular domain of KAI1 is sufficient to trigger cell death. Hence, the hypothesis was that in the primary tumour KAI1 interacts with its ligand on neighbouring cells. If the tumour cells dislodges from the rest of the primary tumour mass, KAI1 is not in contact with its ligand anymore and is, according to the dependence receptor concept, activated for apoptosis.

2 Project objectives

The main objective of this WP was to integrate the dependence receptors into signalling pathways. In particular for KAI1 this meant to determine its ligand and use this factor to prove that KAI1 is a dependence receptor.

3 Project achievements

The HERMIONE project has explored the signalling pathways of DRS by a large panel of techniques (two hybrid screens, split ubiquitin assays, co-imunoprecipitation, siRNA screens,…). We obtained a lot of hits and picked the more promising to investigate more specifically their implication in the signalling.

Regarding KAI1, it has been shown by others to interact with DARC (Duffy antigen receptor for chemokines) in the two-hybrid system and in immunoprecipitations. Hence, we speculated that DARC could be a potential ligand capable of inhibiting KAI1-induced apoptosis. In order to test this, experiments were performed with cotransfections of KAI1 and DARC. In addition, we used a number of DARC-related receptors. While all the chemokine receptors could not exert an effect on apoptosis by KAI1, DARC and HERMIONE LIGAND seemed to decrease KAI-induced apoptosis in cotransfections. Likewise, we could observe this effect in co-cultivations in which we transfected KAI1 or its potential ligands separately, mixed the cells, incubated them and scored apoptosis. In co-transfection experiments in which we investigated the generality of the apoptosis-inhibiting effects

of the KAI1 ligands we observed that HERMIONE LIGAND could reduce apoptosis induced by KAI1 in both Hela and 293T cells, but DARC could do so only in 293T cells. Consequently, in the following experiments we have focused on HERMIONE LIGAND as the interactor that is relevant for KAI1's function as a dependence receptor. We have also conducted additional experiments in which we blocked the interaction between HERMIONE LIGAND and KAI1 using two specific antibodies against the external domains of the metastasis suppressor protein. In both cases have we observed a re-sensitisation to apoptosis by KAI1. For these experiments KAI1 and HERMIONE LIGAND were co-transfected, the respective antibody applied, and apoptosis quantified by FACS. These data are additional evidence that KAI1 is a dependence receptor and that HERMIONE LIGAND functions as a ligand for the dependence receptor KAI1.

Dependence receptors are cleaved by caspases, which is part of their activation for cell death induction. We have detected in the KAI1 sequence a potential caspase cleavage site at the very N-terminus. As this would release a fragment of only 7 residues, which is difficult to detect, we fused a GFP at the C-terminus of KAI1 and discovered that this moiety was removed during apoptosis. Furthermore, this effect could be blocked by the pan-caspase inhibitor zVAD. Moreover, we have generated a number of KAI mutants. One of the, D260A features a subtle point mutation in the aspartic acid that is a requisite residue of caspase cleavage sites. In line with KAI1 functioning as a dependence receptor that is cleaved by caspases, this mutant was inactive for apoptosis induction in MIAPaCa cells and barely active in other cell types. Finally, we detected a DART domain in KAI1, which is a consensus sequence of unknown function that can be found in dependence receptors. This is another indication that KAI1 is a dependence receptor.

New transducers were investigated for RET and KAI1 using the split-ubiquitin two hybrid screening. In the case of RET a specific attention was given to AIP and it was shown that AIP is required for the cell death induced by RET and UNIBO mapped the interaction to the pro-apoptotic domain of RET.

Thanks to a screen using a lentivirus library of 25,500 shRNA, CLB identified genes which inactivation disrupts UNC5H2-induced cell death. CLB has focused on PR65β, a subunit of a PP2A complex and first confirmed that the siRNA encoded by the shRNA sequenced in the surviving clone was efficient in inhibiting PR65β expression and, as a consequence, PP2A phosphatase activity. CLB have then validated that down-regulation of PR65β inhibits UNC5H-induced apoptosis in different cell systems and demonstrated that PR65β interacts with UNC5H2. An important results obtained has been that UNC5H2, PR65β and DAPk (Death-Associated-Protein-kinase) formed a complex upon netrin-1 absence. DAPk is a crucial intracellular protein that mediates cell death induction through a wide spectrum of apoptotic and non apoptotic signals via its serine threonine kinase activity. However, while DAPk is a key player in cell death regulation, very few interacting proteins which function as direct activators of its catalytic activity are known. CLB have then shown that PP2A mediates dephosphorylation of DAPk induced by UNC5H2 and that PP2A activity is required for the dependence receptor activity of UNC5H2 during developmental angiogenesis. Thus, CLB has proposed that the recruitment of PP2A to UNC5H2 allows the activation of DAPk via its loss of phosphorylation and that PP2A activity, via its ability to transduce UNC5H2 pro-apoptotic signal, is important for angiogenesis regulation (Guenebeaud *et al.*, submitted).

Another part of the project investigated the potential role of DCC/neogenin in the nucleus. Indeed it has been demonstrated that DCC/neogenin, are cleaved by γ-secretase and interacts with proteins involved in gene transactivation (Goldschneider *et al.*, 2008, Moll. Cell. Biol.). More specifically, CLB has observed that the intracellular domain of DCC/neogenin issued from the γ-secretase cleavage translocates to the nucleus and triggers specific gene transcription. Chip-Seq are currently being started to unravel the genes transactivated via DCC. The overall view is that this nuclear signalling inhibits the pro-apoptotic signalling of DCC cleaved by caspase (Goldschneider et al., in preparation). Preliminary results concentrated CLB/IMP research on DCC nucleus signalling toward TIP60 protein, a histone acetyltranferase which was already known to play a role in transactivation and has been linked to KAI1. However, we did not observe relevant role for TIP60 in DCC nuclear signaling pathway.

WP2 – Dependence receptors and their ligands in human tumors

1 State-of-the art at project start

There has been evidence for loss of DR expression in numerous tumors and for over-expression of netrin-1 in metastatic breast tumors. Loss of DCC or KAI1, as well as mutations in DCC and RET are associated with bad prognosis, but more data were needed to correlate DR/ligand pair expression to the development of human tumors, especially regarding ligand over-expression and downstream actors of the pro-apoptotic pathway.

2 Project objectives

The objective of WP2 was first to generate tools to study the expression of DRs and identified molecules in human tumors. Situations in which the ligands are over-expressed were of particular interest for WP4. In addition, partners had the project to verify that the signaling pathways deciphered in WP1 are implicated in human tumorigenesis and whether the identified molecules could serve as markers.

3 Project achievements

The HERMIONE project was successful in delivering two new antibodies for netrin-1 detection and for UNC5 genes. One recombinant antibody for netrin-1 (Nora 1) has been developed and was tested for immunohistochemistry and for developing an ELISA kit. The ELISA kit, with a sensitivity of about 1 ng/ml, has been developed and is now validating with the aim of a potential commercialisation. One antibody against UNC5A was developed and tested on paraffin-embedded tissues from colon cancer and matched normal samples, giving a correct immunostaining at the membrane level.

One of the major achievements of the project regarding WP2 has been the identification of netrin-1 expression as specific marker of bad prognosis for a series of tumors. In fact, the expression of UNC5 ligand (netrin-1) was studied by RT-QPCR in a large set of primary tumors and it appears that netrin-1 is up-regulated in about half of lung cancer (Delloye-Bourgeois *et al.*, 2009, JNCI), in a

large fraction of aggressive neuroblastoma (Delloye-Bourgeois *et al.*, 2009, J. Exp. Med) and most of colorectal cancers associated to inflammatory bowel diseases (Paradisi et al., 2009, PNAS).
Also the ligands for KAI1, DARC and HERMIONE ligand, were expected to be up-regulated in certain tumor cells. This would inhibit KAI1 as an apoptosis inducer and would establish an autocrine loop to prevent the tumor cells from undergoing apoptosis. We have investigated tumour tissue microarrays and plotted the number of studies, categorized into different tumour tissues, with a statistically significant up-regulation of the genes. For DARC we observed its up-regulation especially for colon carcinoma. The Oncomine data base was queried in parallel. Its results confirmed the result. The results for HERMIONE ligand were obtained in the same way as for DARC. Also here, the results both from Oncomine and from the microarrays indicate that HERMIONE ligand is up-regulated in prostate cancer. Consequently, the data that we have collected indicate that HERMIONE ligand is up-regulated in a number of tumour tissues with prostate cancer being detected with two different approaches. DARC seems to be up-regulated mostly in colon cancer.

Another task of WP2 has been the search for mutations in DRs receptors and DRs effectors. We have screened one of RET new interactors identified during the project (see WP1), AIP, in pituitary tumors (where mutations in AIP were identified by an independent group) and in patients with familial forms of non-medullary thyroid cancer, but no new mutations were found. Instead the search for DRs mutations led to the identification of new germline variants in UNC5C in colon cancer. These changes in the protein were not found in more than 1000 ethnically matched controls and do not affect protein production but modify the apoptotic effect of UNC5C after being transfected in HEK293 cells. As these changes have an antiapoptotic effect in transfection assays the results thus confirm the role of UNC5C in CRC. In addition, searching for mutations and epigenetic alterations in a panel of breast tumors has revealed that UNC5C promoter is methylated in the tumor tissues, whereas is not in the normal matched tissue, suggesting that UNC5C expression is lost during tumor development.

WP3 – Murine models of tumoral development

1 State-of-the art at project start

Studies with animal models are a necessary step to test our hypothesis on DRs. By the past, few has been done in this context. For example, mice overexpressing netrin in the gut showed a diminution of cell death in the epithelium and an increase in tumorigenesis (Mazelin *et al.*, 2004, Nature) but this study failed to show *per se* that the pro-apoptotic activity of a DR is necessary to limit tumoral development. Therefore, there was a strong need to study additional murine models.

2 Project objectives

To study the impact of loss of DR function on tumour development and to provide proof of concept that loss of apoptosis induction is correlated to tumorigenesis, partners has developed and studied murine models in which the expression of DRs is impaired or the ligand is expressed in an autocrine manner by metastatic cells.

3 Project achievements

The HERMIONE project has allowed the development of numerous mice models (DCC KI D1290N, conditional UNC5H2 DN, PIDD KO, UNC5H3 KO, RET KI D707N) and original crosses (DCC mutant x APC mutant, UNC5H2 mutant x APC mutant, RET mutant x CDK4 mutant). DCC KI D1290N mice exhibit a mutation on the intracellular domain of the receptor which has for consequence to block the exposure of the pro-apoptotic ADD domain. Thus the pro-apoptotic activity of DCC is abolished. Similarly RET D707N mutant is dead for the apoptotic signalling while the positive signalling is unaffected. The conditional UNC5H2 DN mice conditionally express a dominant negative mutant of UNC5H2 that inhibits the pro-apoptotic signalling of the 3 UNC5H –i.e., UNC5H1, UNC5H2, and UNC5H3-. The production of this UNC5H2 DN receptor is controlled by the CRE recombinase. PIDD and UNC5H3 KO mice are respectively classic PIDD and UNC5H3 mutant mice. All these models and their crosses have allowed to study in the one hand spontaneous tumoral development and in the other hand two-hits tumoral development. Along this line, UNIL observed that apoptosis induced by UV irradiations is enhanced in PIDD KO mice and that DCC, UNC5H2 and RET mutant mice do not develop spontaneous tumors. These last results are consistent with the hypothesis that deregulation in dependence receptor pathways is not an initial step in tumorigenesis. Most of the results of the crosses between mice models will be known in the first semester of 2010. As a proof-of concept, the analysis of UNC5H3 mutant gave crucial results.

In these mice CLB first analyzed spontaneous tumour development. However, CLB failed to detect any obvious intestinal tumour development when compared to control mice, hence suggesting that UNC5H3 inactivation in mice is not a sufficient genetic event to trigger tumour initiation. CLB then investigated whether UNC5H3 inactivation affects tumour progression. $UNC5H3^{rcm}$ mice were backcrossed to an $Apc^{+/1638N}$ genetic background. Apc mutations exist in the vast majority of human colorectal tumours, and in mice, specific Apc mutations trigger the formation of tumours in the gastrointestinal tract (mainly low-grade adenomas). CLB demonstrated thanks to this model that UNC5H3 inactivation is associated with enhanced tumour stage in the presence of an Apc mutation, demonstrating *per se* that UNC5H3 loss-of-function is a selective advantage for tumour progression.

The main objective of this HERMIONE project was moreover to demonstrate that the gain of ligand of DRs occurs in tumors and as such provides a survival advantage for tumor growth. CLB thus proposed that netrin-1 could be a marker of metastatic beast cancer and, as predicted by the dependence receptor model, CLB has shown that a cell can escape apoptosis by producing an autocrine loop of netrin-1. CLB also demonstrated that in different models of lung colonisation of syngenic breast tumor cells or of xenograft of primary tuors, disruption of this autocrine loop either by intraperitoneal injection of netrin-1 siRNA or of a decoy DCC receptor (DCC-5Fbn) leads tumor growth/metastasis inhibition (Fitamant *et al.*, 2008, PNAS).

WP4 – Dependence receptors as therapeutic targets

1 State-of-the art at project start

The use of the dependence receptors pathways as a therapeutic strategy is innovative. More specifically the preliminary observation that in metastatic breast cancer (and thanks to WP2 in a

wide range of cancer types) netrin-1 is up-regulated and that this up-regulation is a selective advantage for tumor cell to survive, led the HERMIONE consortium to validate netrin-1 as a target for anti-cancer strategy.

2 Project objectives

The first primary objective of this WP4 was thus to validate netrin-1 as a strong target for anti-cancer strategy. To do so, in different animal models, it was proposed to interfere with netrin-1/receptors interaction using different strategies. The first strategy was the use (as a proof-of-concept compound) of a recombinant ectodomain of DCC (DCC-5Fbn). Thus, this WP aim at identified compounds like blocking monoclonal antibodies and small compounds obtained through robot screens.

3 Project achievements

During the HERMIONE project an important work has been done to provide strong evidence of the interest in disrupting the netrin-1/receptors interaction. Indeed, the HERMIONE project has allowed to work in different contexts of cancers and to test this hypothesis. In breast cancer, CLB demonstrated in xenografted mice model (with either tumoral cells or fresh human tumor) that treatments with DCC-5Fbn (which trap netrin-1) led to the regression and the prevention of metastasis (Fitamant *et al.*, 2008, PNAS). In neuroblastoma, CLB proved with the same approach in chicken and mice *in vivo* models that to inhibit the netrin-1/UN5H2 interaction stopped the progression and the dissemination of the pathology (Delloye-Bourgeois *et al.*, 2009, JEM). In non-small lung cancer, the analysis of xenografted mice treated with siRNA against netrin-1 or DCC-5Fbn shown that tumor growth was inhibited (Delloye-Bourgeois *et al.*, 2009, JNCI). Finally, concerning colorectal cancer, we used mice model for IBD-associated (Inflammatory Bowel Diseases) colorectal cancer and demonstrated titration of netrin-1 expression had a dramatic effect on tumor progression as treatment triggers an increased frequency of low grade adenoma associated with a decreased frequency of high-grade adenoma and adenocarcinoma. Thus, the HERMIONE project has succeed in providing the proof of concept that (i) netrin-1 is therapeutically interesting target and (ii) in animal model disruption of netrin-1/receptor interaction prevent and/or induce the regression of primary tumor or metastasis in neuroblastoma, breast, colorectal and non-small lung cancers.

A goal of this WP4 was then to develop candidate drug to target netrin-1. One of this candidate drug could be a monoclonal blocking antibody. The HERMIONE project has allowed the production of monoclonal antibodies against netrin-1, yet none of them have been shown to display a titrating activity in ELISA assay measuring DCC/netrin-1 interaction. To increase the chance to obtain blocking antibodies against the human netrin-1, antibodies has been developed using a phage display library. Several recombinant antibody candidates have been tested but again was blocking antibody. CLB and CEA dove for the search of small compounds that may interfere on netrin/receptors interaction. CLB, with the help of the tools developed by APOTECH, set up a robust ELISA assay allowing the quantitative measurement of netrin-1/DCC-ectodomain interaction. This robust ELISA was then transferred to the CEA robot platform and the HERMIONE project has

allowed to start a high-throughput screen for small molecules able of inhibiting the interaction of netrin-1 to its receptors. A first screen has been done on a library of 1,120 compounds and 14 hits (inhibition of netrin-1/DCC interaction of at least 10 fold) have been found and are under investigation. A second screen on a 40,000 compounds library has been initiated.

A final goal of the prject was to investigate whether other autocrine loop of ligand could be observed for other DRs. As KAI1 ligand turned of poor interest, we provided the proof of concept on another DR's ligand: NT-3. CLB has worked on the dependence receptor TrkC and its ligand NT-3. CLB observed that NT-3, like netrin-1, is over-expressed in several types of cancers (for example, in Neuroblastoma and breast cancer). CLB initially obtained preliminary results by *in vitro* experiments that showed that the interference with the interaction between TrkC and NT3, with an antibody anti-TrkC which blocks NT3 fixation, induces neuroblastoma cell death. CLB then provided evidence that *in vivo* (in a chicken model and in engrafted nude mice) interference with NT-3/TrkC could be used to limit/inhibit neuroblastoma (NB) progression and dissemination (Bouzas et al., JCI, 2010). We thus provided strong results that allow us to develop another proof of concept for a new potential target: NT-3.

WP5 – Management

1 Project objectives

- Achieve the project's objectives by efficiently using the planned resources
- Fulfil contractual requirements towards the EC
- Develop and facilitate efficient communication between partners and thus optimise indirect benefits of the project

2 Project achievements

The resources of the project were efficiently managed thanks to a regular financial and technical follow up done by the Coordinator with ALMA's support. All the contractual deliverables were sent to the EC respecting the schedule. The withdrawal of SERVIER and APOTECH and the consequent entry into the project of CEA caused the reallocation of tasks and budget and a consequent change in the contract, that were amended and approved by the EC. To ensure an efficient communication, a public web site (http://www.hermione-project.com) was designed and an intranet web platform was developed (https://www.myndsphere.com).

WP6 - Exploitation and Dissemination

The success of HERMIONE relies not only on the quality of the science that was achieved but also on the target validation that was done for further anti-cancer therapy. The netrin-1 patent that was the basis of the work performed in this project has now been discussed with Netris Pharma, a spin-off company of CLB which goals the pre-clinical and early clinical development of drugs based on the dependence receptor concept. An exclusive licence has been given to Netris Pharma in December 2009. During the course of the HERMIONE project, a patent protecting NT-3 /TrkC interference as a promising anti-cancer therapy has been filed by CLB and Netris Pharma is currently discussing an exclusive licence in this patent.

In addition to translational output, The HERMIONE project has also allowed the publications of up to 16 articles in peer-reviewed journals, some in the best journals (Cell, Nature Cell Biology, JNCI, JEM, JCI, …). It also led to numerous international conferences. The communication to a large

public of the work done by European laboratories in both fundamental and translational research is now available thanks to a website (http://www.hermione-project.com) and a leaflet.