

**DC-THERA**

**Final Report 2010**

**Contents**

[2.1 Final Report 3](#_Toc270599541)

[Section 1 4](#_Toc270599542)

[Publishable final activity report 4](#_Toc270599543)

[1. Project execution 4](#_Toc270599544)

[Scientific Background to the Project 4](#_Toc270599545)

[Overview of Project Objectives 5](#_Toc270599546)

[Evolution of the Project 6](#_Toc270599547)

[Work and end results of the project 6](#_Toc270599548)

[Dissemination and use 7](#_Toc270599549)

[Cluster 1. Genomics, Proteomics & Bioinformatics 7](#_Toc270599550)

[Cluster 2. Molecular Cell Biology & Advanced Imaging 9](#_Toc270599551)

[Cluster 3. Immunity, Tolerance & Pre-Clinical Models of Disease 11](#_Toc270599552)

[Cluster 4. Clinical Trials, Immunomonitoring, and Regulatory Affairs 14](#_Toc270599553)

[Cluster 5. Horizontal Activities 16](#_Toc270599554)

[Cluster 6. Strategic Priorities 19](#_Toc270599555)

[2. Dissemination and use 23](#_Toc270599556)

[Section 2 27](#_Toc270599557)

[Final Plan for using and disseminating the knowledge 27](#_Toc270599558)

[Final Management Report 33](#_Toc270599559)

[Final Summary Financial report 33](#_Toc270599560)

[Final Report on the distribution of the Communities Contribution 33](#_Toc270599561)

[Final Report on the distribution of the communities contrbution 33](#_Toc270599562)



Project no. **LSHB-CT-2004-512074**

Project acronym: **DC-THERA**

Project title: **Dendritic Cells for Novel Immunotherapies**

Instrument: **Network of Excellence**

Thematic Priority**: priority Area 1.1.1.i.b. Life Sciences, Genomics and Biotechnology for Health**

# 2.1 Final Report

Period covered: from **01-01-05 to 30-06-10** Date of preparation: **August 2010**

Start date of project: **01-01-05** Duration: **5.5 years**

Project coordinator name: **Prof Jonathan Austyn**

Project coordinator organisation name: **University of Oxford/UK**

Revision: **EC 1st Submission**



**DC-THERA**

**Dendritic Cells for Novel Immunotherapies**

# Section 1

# Publishable final activity report

## 1. Project execution

## Scientific Background to the Project

Dendritic cells (DC) are specialised cells of the immune system that trigger and regulate many different types of immune response. It is convenient to view the position of these cells within the immune system as being placed at the intersection of its two main arms, which respectively mediate innate and adaptive immune responses.

Innate immunity is a phylogenetically ancient form of immunity whose origins can be traced back to the earliest living organisms. According to one view, innate immunity is preoccupied with the perception of ‘danger’, whether as a result of infection or cell or tissue damage. Acting through ancient ‘pattern recognition receptors’ (PRRs) that are encoded in the DNA of both invertebrates and vertebrates, the innate arm of immunity can recognise ‘danger signals’ and trigger specialied responses. These are designed to eliminate different classes of infectious agents, such as viruses, bacteria, fungi and certain parasites, and to aid in repair and healing of damaged tissues.

In contrast, adaptive immunity is a much more recent form of immunity which developed in vertebrates at around the time of the Cambrian Explosion, some 550m years ago. This form of immunity is based on lymphocytes and the specialised receptor molecules they produce, such as antibodies, which are generated by a remarkable and unique process of DNA rearrangement. The lymphocytes and antibodies of adaptive immunity have an astonishing ability to discriminate between the slightest changes in different molecules, for example between mutants of infectious agents, even of the same class. However, they need to be instructed as to whether or not these are ‘dangerous’. If so, the lymphocytes need to be activated and instructed as to what specific types of response they should mount to counter them.

The overarching function of DC, as they are currently understood, is to perceive and discriminate between different types of ‘danger’ and then to activate and subsequently regulate different types of lymphocyte responses. There are two main types of ‘conventional’ lymphocytes: B cells which make antibodies; and T cells comprising CD4-positive ‘helper’ or ‘regulatory’ T cells (different subsets of ‘Th’ or ‘Treg’ cells) and CD8-positive T cells that can develop into cytotoxic T lymphocytes (CTL). DC are unique in being the only, or at least the predominant, cell type that can activate T lymphocytes under most circumstances. Depending on how they have been instructed by the DC, the T cells subsequently become able induce and regulate responses of other cells, such as B cells, or to kill infected cells directly.

Because DC play such crucial and pivotal roles in inducing and regulating adaptive immune responses, there is considerable interest in their potential as therapeutic agents. There are good reasons to believe, for example, that DC could potentially be developed as vaccines to trigger protective immunity against cancers, and potentially against infectious agents such as HIV. Alternatively, their regulatory activites might be exploited to overcome aberrant or unwanted immune responses, such as those that lead to allergies, autoimmune diseases or transplant rejection. The ultimate realisation of these goals (‘dendritic cells as novel immuntherapies’) depends on a translational approach from basic research though pre-clinical models to the clinic, and back again.

## Overview of Project Objectives

As highlighted above, dendritic cell (DC) immunobiology has enormous potential for development of new immunotherapies such as those for cancer and infectious disease. Europe possesses a critical mass of leaders in the field who have pioneered many innovative advances and provided initial proof of principle for these approaches. DC-THERA, ‘Dendritic Cells for Novel Immunotherapies’ is a Network of Excellence (NoE) established under the European Commission’s Sixth Framework Programme (FP6). Its original scientific and technological [S&T] objectives were to encourage and facilitate the translation of genomic, proteomic and bioinformatic information, with knowledge from molecular cell biology and pre-clinical models, into therapeutic endpoints focussing on new and continuing clinical trials of DC-based therapies for cancer, and to a lesser extent HIV infection and AIDS. Its aims were originally to promote the integration of the activities of 32 partners working in the above areas (comprising 26 groups of scientists and clinicians with 6 SMEs in 9 member states of Europe); it additionally incorporated a further 43 groups as Associated Partners/Third Parties of the Network over its lifetime (including several groups from new and future member States). ***All DC-THERA participants are listed at the end of this section.***

Over its lifetime DC-THERA created an infrastructure to facilitate collaborative working between its Partners and Associated Partners/Third Parties, and to capitalise upon, and enhance, their collective and complementary expertise and resources. Towards this end, four thematic S&T Clusters were originally defined [Clusters 1-4]. A fifth focussed on horizontal measures including the development of technological platforms, provision of new education and training opportunities, incorporation of Associated Partners/Third Parties, dissemination of information, and Network management [Cluster 5]. Following a mid-term review of the Project, new Strategic Priorities were identified for the remainder of the Contract, requiring the creation of an additional, cross-thematic Cluster [Cluster 6]. Specific milestones and deliverables were defined for each of the 20 Work Packages within these Clusters and, by the end of the project, the majority of these were achieved and completed. Just less half of the Network’s entire budget of € 7.6m over 66 months was allocated to its individual Partners, while the remainder was used to support the horizontal activities in Cluster 5 and the later Strategic Priorities in Cluster 6. This overall budget distribution is appropriate for a Network of Excellence, as opposed to other types of project that are, for example, designed primarily to fund the research and clinical activities themselves.

## Evolution of the Project

The DC-THERA Contract commenced January 1st 2005 and ended June 31st 2010. The project can be viewed as having evolved in two main phases: the first from its commencement to its mid-point in about mid-2007; and the second from the mid-point to the end of the Contract.

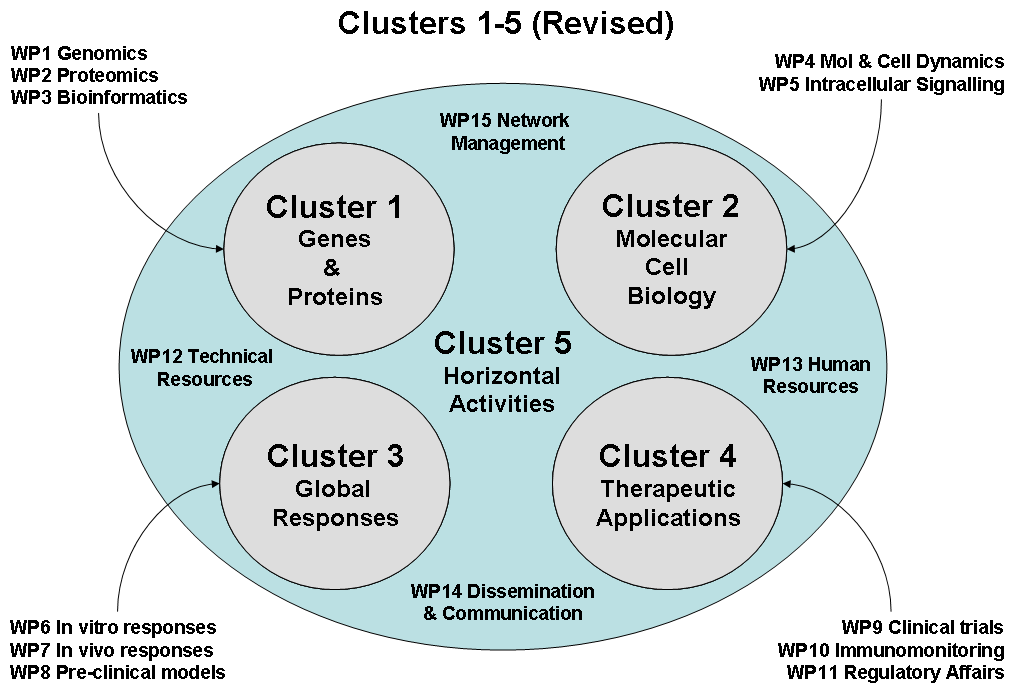
In its first year (2005), the Network made considerable progress in beginning to implement its infrastructural measures in Cluster 5, and the majority of these were consolidated during the second (2006). Then, in the third period (2007), further progress was made towards meeting the Project’s scientific and technological (S&T) objectives in Clusters 1-4. By the mid-point of the Project (mid-2007), the last of its planned horizontal activities in Cluster 5 had been implemented. Following an internal mid-term (2007) review of the Project, several new Strategic Priorities were identified (in addition to the continuation of many of the activities in Clusters 1-5). These were designed, in Cluster 6, to (i) provide a new focus for the Network’s activities; (ii) further integrate the different areas being covered by the Project; and (iii) provide new opportunities for collaborative working by its participants to contribute to its overall aims.

By the end of the project all milestones and deliverables in 18 of the 20 Work Packages had been achieved; the few that have not yet been achieved in the 2 remaining Work Packages are expected to be achieved and completed by end 2010.

## Work and end results of the project

The four S&T clusters that were initially defined encompass studies of genomics and proteomics (Cluster 1), molecular cell biology (Cluster 2), immunobiology and pre-clinical models of disease (Cluster 3) and therapeutic applications including clinical trials (Cluster 4). Crossing these, a series of horizontal activities (Cluster 5) was put in place to increase the technical and human resources available to the Network, to facilitate dissemination of information and communication both within and outside of the Network, and to manage the Joint Programme of Activities as a whole. These Clusters, and their component Work Packages (WPs) are schematically illustrated below (‘revised’ relates to relocation of WP6, originally in Cluster 2, to Cluster 3).

An outline of the work and examples of end results of each of these Clusters and their component Work packages is discussed below; Cluster 6 is described later. Clearly, in a report of this type, one cannot do justice to the vast amount of work that has been undertaken by its 32 Network partners (and its 43 Associated Partners/Third Parties) over a total period of 66 months. Nor, in fairness to all, will we name specific individuals. Instead we will highlight just a few of the Networks’ achievements in the context of the ‘state of the art’ in the field of DC immunology. In addition, we will try pull together some themes that run through some of the different areas of work to emphasise a key goal throughout this project which has been, wherever possible, to facilitate the translation of basic findings from the bench to the bedside, and back again.



## Dissemination and use

### Cluster 1. Genomics, Proteomics & Bioinformatics

DC are highly plastic cells. They can perceive different types of ‘danger’, and discriminate between different classes of infectious agent (e.g. viruses, bacteria and fungi), normal versus abnormal forms of cell death (apoptosis and necrosis respectively) and other signals from their environment (e.g. cells responding to tissue damage). Crucial to this type of recognition are ‘pattern recognition receptors’ (PRRs). Signalling through some of these, in response to different types of ‘danger’ can lead to rapid and marked changes in DC function, in a process collectively termed DC activation or ‘maturation’. These typically endow the DC with new capabilities to activate and regulate different types of T cell responses. In other cases, different PRRs promote the uptake (endocytosis or phagocytosis) of small microbes by the DC in cytoplasmic vesicles (endosomes or phagosomes) which fuse with preformed organelles (lysosomes) that deliver toxic, degradatory and other molecules into the vesicle to kill and degrade the microbe. Underlying DC maturation and their functions is the differential expression of a vast number of genes, and further complex modifications of the proteins they encode in a highly regulated manner. Understanding these can provide fundamental insights into the regulation of DC functions and potentially how they can be modulated for therapeutic purposes.

#### Work package 1. Genomics

Genomics, involving transciptional profiling at the mRNA level, can reveal perturbations in expression of a vast number of genes in response to any given stimulus; this is the focus of WP1.

There are in fact different populations of ‘classical’ DC that stimulate T cell responses in the manner noted above, in addition to other types of DC that can be generated in culture and which are typically used, for example, for DC-based vaccination. Over the course of this project, transcriptional profiling of DC generated from progenitors in culture has provided insights into how gene expression is regulated in response to defined stimuli, such as purified microbial components or a ‘cytokine cocktail’ that is typically used to mature DC for clinical purposes. It has also enabled a deeper understanding of the specialised responses and functions of different DC populations that are present in vivo. For example they have been applied to the different subsets of ‘classical’ DC, such as CD8-negative versus CD8-positive DC isolated from mouse lymphoid tissues. As a consequence, some of the key genes that may be involved in their specialised functions have been identified, such as ‘cross-presentation’ of antigens by the latter subset which may be crucial for DC to be able to activate CTL if the DC are not, themselves, infected.

These studies have required a substantial number of hybridisation experiments using ‘Affymetrix’ gene chips, particularly for studies with DC from mouse and, to a lesser extent, rat. The same was initially true for human DC although, later in the project, the alternative use of ‘Illumina’ gene chips became routine [see Cluster 6]. Technological developments had to be made and introduced to extend the power and sensitivity of these approaches. For example new techniques to enable processing and analysis of very small [~2 ng] samples of mRNA has considerably increased the repository of information that could be obtained form such studies. Real-time verification of gene expression was performed by RT-PCR, and further validation was carried out by comparing changes in mRNA expression with those of corresponding proteins, for example by using multiplex analysis to quantify the secretion of multiple different cytokines by DC in response to different stimuli.

Transcriptomic profiling has also been used to compare the transcriptomes of DC and macrophages. It has also been applied to modulated DC, for example after exposure to specific kinase inhibitors, in order to understand the role of individual signalling components in their responses to different stimuli (cf. WP5). It has been further extended to T cells that have been co-cultured with DC in order to identify new markers of T cell polarisation (for example Th1 versus Th17 cells; cf. WP8). Ultimately the studies in this area (WP1), which continued throughout the project, were considerably enhanced by further initiatives that were later introduced in Cluster 6 (WP16-18; below).

#### Work package 2. Proteomics

Proteomics can reveal perturbations in a large number of proteins, both in whole cells or in subcellular fractions of those cells such as the cytosol or cytoplasmic organelles; this is the focus of WP2. Hence genomics and proteomics are complementary techniques that together can help build a more complete picture of dynamic changes in gene and protein expression following stimulation, and of how these are modulated by different stimuli. At the commencement of this project, however, a major problem was that some of the available proteomic techniques, particularly ‘SILAC’ labelling of cells, were suboptimal for use with DC. Hence an new approach to enhance proteomic analysis of DC had to be developed, involving a novel label-free system. Ultimately this has enabled proteomic analysis of DC to a depth of in excess of 5,000 proteins. A major effort has been towards proteomic mapping of different intracellular organelles of mouse or human DC, including phagosomes and lysosomes, and in comparing some of these with those of macrophages. Proteomic analysis has also been carried out on exosomes that are liberated from DC, and which have been used to deliver antigens to DC in some DC-THERA clinical trials (Cluster 4, WP9). This area of work also led to the first, in depth, systematic study of the proteome of ‘classical’ DC subsets isolated from mouse lymphoid tissue and of ‘non-conventional’ plasmacytoid DC which have different or additional immunological properties to the classical cells. This major collaborative project has revealed startling new insights into the specialisations of these cells. For example, it was discovered that the CD8-positive subset of DC (noted above) essentially lacks the PRRs that can normally sense viral infections, and may provide a mechanistic explanation as to why these cells are so efficient at cross-presenting antigens.

#### Work package 3. Bioinformatics

Increasingly sophisticated bioinformatic tools are needed to manage, store, retrieve and analyse the enormous amount of data that are generated by transcriptomic and proteomic analysis; this is the focus of WP3. Bioinformatics renders these data more interpretable in a biological context, and potentially exploitable in a therapeutic framework. In the early part of the project much work was expended on implementing and enhancing the RDFScape bioinformatic platform for more detailed analysis of ‘Affymetrix’-based transcriptional profiling. In the second half, an entirely new bioinformatic system was developed, particularly for comparison between different microarray experiments performed on the ‘Illumina’ platform, and in relation to newly-defined signalling pathways in DC. These tools have, in another collaborative project, enabled the transcriptomes and proteomes of human DC exposed to a defined maturation stimulus to be compared simultaneously, this being the ‘cytokine cocktail’ used by several partners to mature DC that are used in clinical trials of cancer patients (Cluster 4, WP9; Cluster 6, WP18). These studies revealed that there was very poor, if any, correlation between individual changes in mRNA expression and those of the corresponding proteins. However, substantial similarities between them by using the technique of pathways-based analysis’ (instead of looking at changes in expression of individual components). From this it has been possible to identify key maturation markers that may contribute to the design of more effective DC-based vaccine strategies in the future. Because of the power of pathways-based analysis in helping to interpret ‘omics’ data, this became a major focus in the second half of the project (Cluster 6, cf. WP16-WP18)

### Cluster 2. Molecular Cell Biology & Advanced Imaging

The dynamic, intermolecular interactions that occur in different intracellular compartments and at the plasma membrane of the cells are central to the specialised functions of DC. So to are the intercellular interactions and two-way communication between ‘classical’ DC and different cell types of both innate and adaptive immunity. The former include plasmacytoid DC and NK cells, and ‘non-conventional’ T cells such as NKT cells and gamma delta T cells. The latter include the ‘conventional’ alpha-beta T cells, and potentially B cells. Visualisation of intermolecular interactions in living cells, and of intercellular communications in living tissues, can provide deep insights into immunological and pathological processes in which DC may play a pivotal role. Furthermore, the rational manipulation of these processes can potentially modulate the functions of DC and other cell types such that they can be exploited to enhance or suppress aberrant or unwanted immune responses in a therapeutic setting.

#### Work package 4. Molecular and cellular dynamics

Development of wide variety of sophisticated imaging techniques now enables the visualisation and localisation of molecular assemblies in living cells, and of cellular interactions in living tissues, both in real time. This is the focus of WP4. For example near-field scanning optical microscopy (NSOM) has enabled the visualisation of individual molecules of DC-SIGN (a PRR) clustered together in nanosized domains, randomly distributed over the surface of DC. Further information about the mobility aof molecules, and distances between them, can be obtained through the techniques of fluorescence recovery after photo-bleaching (FRAP) and fluorescence resonance energy transfer (FRET) respectively. For these types of work, which have been applied to several key molecules of DC in this project, a variety of fluorescently-labelled probes had to be designed and synthesised, ultimately enabling the visualisation of up to 13 individual dyes to a resolution of ~6nm.

Other imaging techniques such as confocal and multi-photon microscopy have been used to visualise cellular interactions in tissues such as lymph nodes. The latter technique, for example, revealed that serial brief interactions between DC and CD8 T cells were normally required for that latter to become activated and develop into cytotoxic cells, whereas more stable and longer-lasting interactions with DC were needed to them to secrete specific cytokines. In lymph nodes draining tumours however, FoxP3-positive Tregs, that are apparently induced as tumours develop, actually kill the DC, and potentially limit the activation of CD8 T cells. This technique has also been used to visualise the interactions between DC and NK cells, and between a subset of macrophages and NKT cells, in lymph nodes. Other techniques such as magnetic resonance imaging (MRI) have been used to trace the fate of DC, administered as vaccines to cancer patients, and their migration into regional lymph nodes. Such studies confirmed that only a very small mumber of the administered cells actually gained access to the nodes. These insights have have lead to new studies in which, following proof of principle in mice, human DC have been modified to enable them to reach lymph nodes in high numbers after injection into the blood (cf. WP5).

#### Work package 5. Modulation of genes and proteins

The development of technologies has increasingly enabled genes to be ‘silenced’ within DC, or for new genes and proteins to be introduced into them, thus endowing the cells with specific new functions. These include molecular components that are involved in intracellular signalling pathways from receptors at the cell surface to the nucleus, thus modulating gene expression, or to other cellular components such as the cytoskeleton which is involved in both phagocytosis of microbes by DC and movement of the cells, for example when they migrate from peripheral sites into lymphoid tissues to induce adaptive immune responses. This is the focus of WP5

Specific kinase inhibitors have been used to interfere with some key intracellular signalling pathways (see WP1). These have, for example, enabled the induction of costimulatory molecule expression to be uncoupled from cytokine secretion in DC (see Cluster 3). Newly developed ‘gene silencing’ techniques using siRNA or shRNA (introduced by lentiviral vectors) have also used to inhibit components of intracellular signalling pathways in DC, with an efficiency of 70-80%. In other approaches, novel molecules have been expressed in DC after the introduction of mRNA encoding them into these cells; these include those in the ‘TRI-MIX’ cocktail (Cluster 6, WP18). Furthermore genetically-engineered mice (e.g. transgenic mice lacking specific kinase genes or other signal transduction components) have been constructed and studied. The impact of these approaches on the immune function of DC has been further investigated (cf. WP6, WP7) to provide both fundamental insights into the functions of individual molecules, and how they may potentially be used to modulate DC function for therapeutic purposes.

### Cluster 3. Immunity, Tolerance & Pre-Clinical Models of Disease

[‘Conventional’] DC populate all peripheral, non-lymphoid tissues where infection typically occurs. As noted above, they express specialised pattern recognition receptors (PRRs) at the cell surface and in intracellular compartments such as endosomes (in which microbes are internalised) and the cytosol (where viruses typically infect). These PRRs include members of the Toll-like receptor (TLR) and C-type lectin receptor families (e.g. DC-SIGN, above). Signalling through some of these leads to the differential expression of specialised molecules at the cell surface and the secretion of different patterns of soluble molecules such as cytokines and chemokines, both of which enable communication with or recruitment of other cell types. Hence there is considerable cross-talk between DC and innate cells in peripheral sites in the early stage of immune responses.

DC express a diversity of receptors that are involved in uptake of antigens and delivery to specialised intracellular compartments and/or further modulation of their properties. Some of these are crucial for antigen processing and presentation. This first involves the internalisation and/or degradation of microbial components and/or microbes that may have invaded peripheral sites. Peptides derived from them can then be loaded onto MHC molecules. These peptide-MHC complexes are then expressed at the cell surface where, after the DC migrate into lymphoid tissues, they may be detected by the specialised T cell receptors of ‘conventional’ (alpha-beta) T cells. Second, in response to ligation of different receptors, DC may also express specialised ‘costimulatory’ molecules that are required for conventional T cell activation. Further signals, such as specific cytokines the cells produced, then reprogramme the T cells which develop into specialised subsets of helper T cells (Th1, Th2, Th17), or CTL, depending on the type of infection that has occurred. In other cases, however, DC may induce regulatory T cells (Treg) that suppress immune responses, or otherwise delete or inactivate T cells, resulting in immunological unresponsiveness or tolerance. Hence DC contribute substantially to determining the balance between immunity and tolerance, for example in response to infection or to normal components of the body respectively, and ultimately between health and disease. By modulating this balance, it is increasingly possible to generate DC with different properties that can be used in therapeutic settings.

#### Work package 6. Understanding immune responses *in vitro*

While much is known about how DC induce and regulate immune responses, there are very considerable areas of uncertainty, and many unknowns. Much fundamental scientific knowledge can be obtained from studies of individual cell types, such as DC isolated from lymphoid tissues or generated from progenitors in culture, and of the cross-talk between these and other purifed cells with which they are co-cultured. This is the focus of WP6.

A major focus has been towards the discovery or characterisation of new or known receptors of DC that are involved in uptake of different types of antigens, and in stimulation of different types of DC function. A very considerable effort has, for example, elucidated some of the key signalling pathways from PRRs such as the Toll-like receptors, typically involved in sensing viral or microbial infection, and C-type lectin receptors, such as dectin-1 and dectin-2 which sense fungal infection (Cluster 6, WP16). This area of work has also led to the discovery of the first known receptor that is involved in recognition of components of necrotic cells, DNGR-1 (another C-type lectin receptor). It is now clear that this molecule, which is expressed by CD8-positive mouse DC is involved in cross-presentation of antigens from necrotic cells, and further work has demonstrated that its expression of is restricted to a subset of human DC that may thus represent an analogous subset to that in mouse.

A further focus of WP6 has been towards the elucidation of the antigen processing and presentation pathways of DC and of how these are regulated. These have, for example, provided detailed insights into the processes through which peptides from different types of antigens (e.g. viruses or phagocytosed microbes) can be delivered to MHC class I molecules for cross-presentation to CD8+ T cells [CTL], and how these pathways differ in different types of DC. They have also revealed the importance of another presentation pathway, in which lipid-containing molecules are loaded onto CD1 molecules, enabling cross-talk between DC and an ‘invariant’ subset of NKT cells. In addition, much work has investigated how DC are able to stimulate different types of ‘conventional’ T cell responses in culture, using a variety of different immunological assays *in vitro*, for example after delivery of antigens by different routes (e.g. via different receptors) or after exposure to different microbes or fungi, or defined components from these. This has provided new information into the control and regulation of Treg versus Th1 responses, and Th1 versus Th17 responses. Potentially, this knowledge could be used to inform new therapeutic strategies, for example to overcome the suppressive environment that is typically induced by growing tumours, or the immunological evasion machanisms that are used by different types of pathogen.

#### Work package 7. Modulation of imune responses in vivo

While much can be learnt from in vitro studies, in vivo studies are essential in order to translate this knowledge to the level of the intact immune system. This is the focus of WP7. These approaches enable the impact of experimental manipulations in vitro to be investigated further, and may suggest new therapeutic strategies that can be tested later. In some cases, for example, DC that have been modulated in culture can be administered to animals (an *ex vivo* approach, as used in many clinical trials of DC-based vaccination) and the impact on immune responses can be assesed using a variety of assays. Alternatively different agents can be targetted to DC in vivo for the same purpose.

The impact of targetting antigens to DC through different C-type lectin receptors (e.g. DC-SIGN and DNGR-1; above), opsonic receptors such as Fc gamma receptors (which bind antibody-coated antigens), and other routes, has been assessed and compared. In general, antigen targetting has been found to enhance considerably both the quantity and quality of the immune responses that are subsequently generated, for example by promoting robust CTL responses or different types of antibody responses. It was also discovered that adminstration of long peptides derived from antigens of choice, together with an adjuvant, leads to selective presentation by DC in vivo and greatly enhanced T cell responses in mice. This approach has now been translated into clinical trials of patients with vulvar neoplasia, with remarkable therapeutic success (Cluster 4, WP10).

Our increased understanding of the functions of different types of costimulatory molecules has also enabled some of these to be articially expressed in DC, to modulate their in vivo function. One particularly important development, for example, has been the demonstration in mouse models that the introduction to DC of mRNAs encoding a defined antigen, plus a constitutively active (mutated) TLR4 molecule and two specific molecules involved in costimulation of T cells (CD40, CD70) greatly enhances CTL responses against the antigen of choice. After development of this technique for use with human DC, this ‘TRI-MIX’ approach is now also being systematically explored in clinical trials of advanced melanoma patients (Cluster 4, WP9; Cluster 6, WP18).

Over the course of this project, techniques were also developed to modulate the migration patterns of DC for therapeutic purposes. Migration of ‘classical’ DC from peripheral sites into lymphoid tissues such as lymph nodes is crucial for the induction of T cell and other adaptive immune responses. To be effective as vaccines, it is also crucial in the case of DC that are administered to cancer patients, for example by injection into the skin. However, fundamental MRI studies that were carried out in the latter setting revealed that very few DC actually left the injection site (cf. Cluster 2, WP5). To overcome this problem, mRNA encoding a chimeric adhesion molecule, based on some that are expressed by lymphocytes and which enable them to enter lymph nodes directly from the bloodstream, were introduced into mouse DC. After injection into the blood, it was found that these modified cells were indeed able to enter lymph nodes in large numberes and subsequently stimulated potent immune responses. This approach is also being translated into clinical trials (Cluster 4, WP9; Cluster 6, WP18).

#### Work package 8. Pre-clinical models of disease and therapy

Pre-clinical animal models are invaluable for dissecting the role of individual immunological components in mediating disease, and for exploring new therapeutic strategies that might ultimately be translated to the clinic. This is the focus of WP8. Several participants with DC-THERA have established or have access to robust mouse models of cancer, including transplantable and spontaneous tumours; infectious diseases including those caused by specific viruses and bacteria; allergies including asthma; and autoimmune diseases including organ-specific conditions. Further insights have been gained from related studies in genetically-engineered (transgenic) mice. Collectively, these pre-clinical studies have complemented the clinical studies that have been widely undertaken in cancer patients and, to a lesser extent, in HIV-infected individuals.

At a very basic level, studies under normal, steady state and inflammatory conditions have enabled a new DC progenitor to be identified that can develop into both ‘classical’ and plasmacytoid DC. In cancer models, a transplantable melanoma model has been extensively used to gain insights into the function of suppressive tumour-infiltrating cells and the generation of deleterious ‘induced’ regulatory T cells (Treg), viral vectors encoding tumour antigens have been used to delay or prevent carcinogenesis, and DC-based therapies in a spontaneous breast cancer model are also being investigated. In infectious disease models, profound insights have been obtained into the importance of innate immune responses and/or the roles of different T cell subsets (e.g. Th1, Th17, CTL) in defence against infection or disease caused by viruses such as Ectromelia and LCMV as well as two clinically-important species of intracellular bacteria, *Mycobacterium tuberculosis* and *Listeria monocytogenes*, the causative agents of tuberculosis and listeriosis. Similar studies have been carried out in inflammatory disease models such as a Th1-biased model of inflammatory bowel disease (and another of multiple sclerosis), and a Th2-biased model of airway hyper-responsiveness and asthma. In the former case it was discovered that treatment with a therapeutic antibody could ameliorate disease, partly by inducing beneficial regulatory T cells; in the latter case, it was found that airborne antigens were efficiently transferred from lactating mothers to the neonate in the milk and that this induced protective tolerance, again in part through induction of Tregs.

### Cluster 4. Clinical Trials, Immunomonitoring, and Regulatory Affairs

Despite the success of *prophylactic* vaccination to protect individuals before exposure to pathogenic infections [smallpox, polio etc] there are currently no *therapeutic* vaccines available that can be used to treat patients with diseases such as cancer or HIV infection. This represents a considerable challenge. However the ability to grown large numbers of human DC from progenitors (e.g. monocytes) in culture, deliver antigens and expose the cells to defined stimuli to enhance their immune functions, and then administer the cells back to the individual offers, for the very first time, a really new therapeutic approach. In addition to these *ex vivo* DC-based strategies, our increased knowledge of how to target antigens to DC *in vivo* offers additional or alternative approaches.

#### Work package 9. Clinical trials

Many attempts are now being made to develop more effective DC-based vaccination strategies to treat advanced cancer patients with a variety of solid or haematological malignancies and, in some cases, HIV-infected individuals. Current and new clinical trials are systematically investigating the efficacy of different therapeutic regimes in relation to the induction of protective immunity and stabilisation or cure of disease; these include comparison of DC treated with different types of antigen or exposed to different maturation stimuli, and the doses, routes and schedules of DC administration. This is the focus of WP9.

Over the course of this project, approximately 16 clinical trials have been performed using DC-based vaccination, and about 13 of these have been completed; these include smaller pilot studies to evaluate the induction of immune responses and larger stage III/IV clinical trials to evaluate clinical efficacy. These trials have focussed mainly on treatment of advanced cancer patients including those with melanoma, glioma, carcinoma of the colon, kidney or pancreas, and chronic lymphocytic leukemia; clinical trials in HIV infected individuals have also been conducted.

The main approach has been to grow DC from monocyte precursors in culture from each patient and to load them with different forms of tumour-associated antigens, for example as peptides, or as mRNA encoding these antigens, or in exosomes. After maturation, typically with a cytokine cocktail, the DC are injected back into the patients, for example into the skin (intradermally or subcutaneously) or into lymph nodes by ultrasound guidance. Alternatively, patient DC have been fused with tumour cells and the cell hybrids have been administered instead. These ‘ex vivo’ forms of treatment have proven to be safe and well-tolerated, with very few side effects and no cases of autoimmune disease being reported (other than vitiligo associated with regression of melanoma). In addition, well-documented cases of tumour regression have been reported in a number of patients following treatment. Remarkably in some centres at least 25% of advanced melanoma patients who have failed all other forms of conventional therapy but have been vaccinated with DC live for >24 months (compared to a life expectency of just a few months) and some are now surviving more than 5-7 years.

A different approach has been taken to vaccinate women with vulvar intraepithelial neoplasia (VIN) which is associated with human papillomavirus 16 (HPV16). It was found that vaccination with long peptides derived from the sequence of the E6 and E7 proteins of this virus, with adjuvant, lead to remarkable clinical responses with complete regression of the lesions in some cases and a complete clinical response in about half of the treated patients. Complementary studies in mouse models (cf. WP8) have provided evidence that the delivery of long peptides in this way targets DC in vivo, which subsequently stimulate immunity, whereas delivery of short peptides targets other cell types which are unable to express costimulatory molecules and induces tolerance. This clinical trial, together with those noted above and earlier [cf. WP7) thus provides a clear example of the type of iterative approach (from bench to bedside and back again) that has been promoted in this project. It has also been possible increasingly to introduce standardised tools and protocols, thus enabling the DC-THERA multi-centre clinical trial to be undertaken (Cluster 6, WP16).

#### Work package 10. Immunomonitoring

Despite some promising results from multiple clinical trials there are currently still no immunological correlates that can inform the treatment of choice for the best therapeutic outcome. This, in part, is the purpose of immunomonitoring, the focus of WP10. Different immunological assays can now be used to evaluate different forms of immunity in treated patients in attempts to determine which type is most beneficial in which setting. In addition, immunomonitoring provides a means of evaluating and comparing the quality and quantity of immune responses induced by different DC-based or other treatment modalities, to decide which may be the most efficacious.

Over the course of this project, different approaches have been developed or refined to provide increasingly detailed insights into the immunological responses that can be induced by DC-based or other vaccination strategies, providing basic scientific information in the human *in vivo* setting on one hand, and informing the future design of better vaccines on the other. One important approach has been to study the T cells that infiltrate the skin during delayed type hypersensitivity reactions (DTH, induced by injection of e.g. antigen-treated DC into the skin after initial vaccination). It is now clear, after studying more than 200 patients in one particular centre, that this type of analysis provides a better correlation of clinical course than similar studies of T cells isolated from the blood. Another approach has been to isolate CD4 (‘helper’) or CD8 (‘cytotoxic’) T cell clones from vaccinated patients and to study their antigen specificities using MHC tetramers or pentamers, or libraries of overlapping peptides. In addition, new techniques have been developed that allow, for the first time, accurate quantification of Tregs (a PCR based assay based on hypermethylation of the FoxP3 gene) and, increasingly, of the induction of CD4 or CD8 memory T cells (which are crucial to prevent recurrence of disease). As for the clinical trial protocols, a set of standardised immunomonitoring protocols is being used to monitor patient responses in the multi-centre trial (WP16).

#### Work package 11. Regulatory affairs.

Clinical trials are enormously time-consuming, labour intensive and expensive to conduct. Part of this relates to the need to comply with national and European regulatory guidelines, which are designed to protect both the doctor and the patient. This area, regulatory affairs, is the focus of WP11. Throughout the project this work has focussed on the preparation of clinical trial protocols, with accompanying administrative information (case report forms etc) to enable these trials to be undertaken. In addition, in the first half of the project there was significant (and ultimately successful) dialogue with the EMEA, including several meetings with DC-THERA representatives, in relation to a draft ‘Guideline on Human Cell-based Medicinal Products’. In the second half of the project one of the partners undertook a comprehensive, detailed and critical review of all DC-based clinical trials conducted to date or in progress, noting all relevant parameters relating to treatment and subsequent outcome measures; another partner will update this shortly. This database will now be linked to the DC-THERA Knowledge Portal (Cluster 6, WP20) by the end of 2010 and made available to all interested parties.

### Cluster 5. Horizontal Activities

DC-THERA comprises a large number of partners with complementary expertise and resources in different European countries. However, relatively few formal collaborations had been established between them at the start of the project. An overarching aim of DC-THERA was to integrate the activities of these partners, and to promote collaborative working between them, in order to build a critical mass of scientists, clinicians and SMEs working in the area of DC immunobiology within Europe, and to enhance both their individual and collective contributions to the field. A series of horizontal measures was therefore put in place in a fifth Cluster to support these aims.

#### Work package 12. Technical resources

One main objective was to increase the available technological resources and to make these more widely available to Network participants; these also included shared tools and standardised protocols. This was the focus of WP12. Early in the project, three key technological platforms were established, in genomics (e.g. for transcriptional profiling, see Cluster 1), advanced imaging technologies (e.g. for visualisation of molecules and cells, see Cluster 2), and for cell therapeutics (e.g. DC-based vaccination strategies, see Cluster 4). Towards the mid-point of the project a proteomics platform was established (see Cluster 1) and enhanced by the recruitment of two Associated Partners/Third Parties with specific expertise and resources, and in the second half of the project the genomics platform was strengthened by incorporating a further partner. A initial investment by DC-THERA enabled these platforms to recruit additional personnel (e.g. to process samples for gene chip analysis, or to help run a GMP facility) or to develop shared tools (e.g. fusion proteins for microscopic analysis). In return, they provided technical services, workshops and training courses for Network participants (see WP13).

The genomics platform based in Milan, IT, and later incorporating a group in Oxford, GB, enabled sample processing and analysis by ‘Affymetrix’ and ‘Illumina’ technologies respectively. The advanced imaging platform was established between four centres that respectively provided expertise and facilities for high resolution molecular imaging techniques and MRI of patients in Nijmegen NL; MRI of mice in Erlangen DE; two photon microscopy in Paris FR; and correlation spectroscopy in Marseilles FR. The proteomics platform, with state-of-the-art mass spectrometric techniques and instrumentation was originally established in DK but later relocated to Munich DE, and incorported groups from Nijmegen NL and Edinburgh GB. The cell therapeutics platform, based in Erlangen DE, provided GMP facilities for generation of DC-based vaccines for clinical use, and later for mRNA synthesis in relation to the DC-THERA multi-centre clinical trial which was initiated towards the end of the Contract (see WP18). Over the course of this project the platforms made an enormous contribution to the provision of technical services to, and/or workshops and training courses for, other Network participants including Associated Partners/Third Parties (WP13). In the second half there was, in addition, a further series of intensive training sessions for curators involved in the pathways-based initiative in WP16 (see Cluster 6).

#### Work package 13. Human resources

A further objective was to increase the human resources of the Network by providing new education and training opportunities for all, including newly recruited young investigators. Towards the same aim, the Network additionally incorporated a large number of Associated Partners/Third Parties who both contributed to, and benefited from, the Network’s activities. This was the focus of WP13.

To enhance its education activities, while furthering collaborative working between partners, over the course of the project DC-THERA created 14 new PhD studentships, funded for a total of 41 person years. These directly integrated 23 of the Network’s Partners and one Associated Partner/Third Party in 14 different collaborative research projects. By the end of the project, 10 of these students have successfully completed their research projects and have been awarded degrees; the remaining 4 will complete by mid-2011. To enhance its training activities, over the first half of the project, the genomics, advanced imaging and cell therapeutics platforms delivered high quality workshops or training courses, every quarter-year on average. These included lectures and seminars followed by experimental sessions in the former two cases, and intensive two-week training courses in the latter.

A partner in Zurich, CH, also developed a series of DC-THERA Graduate Schools, ‘DcCrest’, five of which were held on an annual basis in Celerina, St Mortitz CH. These were facilitated by a small number of faculty members (and, in some cases, international experts in the field from Australia) but were largely run by the 25-30 postdoctoral fellows and graduate students who were attending the School. In addition to presenting their own work in lectures, and attending group discussions in the evenings, these young researchers worked together before and during the meetings on preparation of grant proposals around specific topics that were then critiqued, with feedback, by the faculty. A Visiting Scholars Scheme was also established to encourage mobility of PIs and more senior scientists or clinicians in two-week to two-month visits to other participants.

It should be stressed that all of the above activities (except for the joint PhD studentships which were established early in the project) were made available to the Associated Partners/Third Parties, the Network paying for all allowable expenses relating to their participation. A total of 43 were incorporated into the Network over its lifetime. This was in response to two open calls for Associated Partners/Third Parties that were made by DC-THERA in the first half of the project; a few were also specifically targeted for recruitment in the second half of the project, such those with specific expertise or resources that were currently unavailable to Network partners. This initiative was of great mutual benefit to both DC-THERA and many Associated Partners/Third Partners.

#### Work package 14. Communication and dissemination

The further objective was to provide an infrastructure to promote communication and dissemination of results both within DC-THERA and with others outside of the Network. This was the focus of WP14. A website was initially developed early in the project to publicise and promote the activities of DC-THERA to the wider arena. This was later supplemented by the development of an intranet to facilitate communication between all partners and with DC-THERA coordination, and later by an extranet to broaden this to all Associated Partners/Third Parties. These initiatives facilitated communication and dissemination of information both within and across the Network.

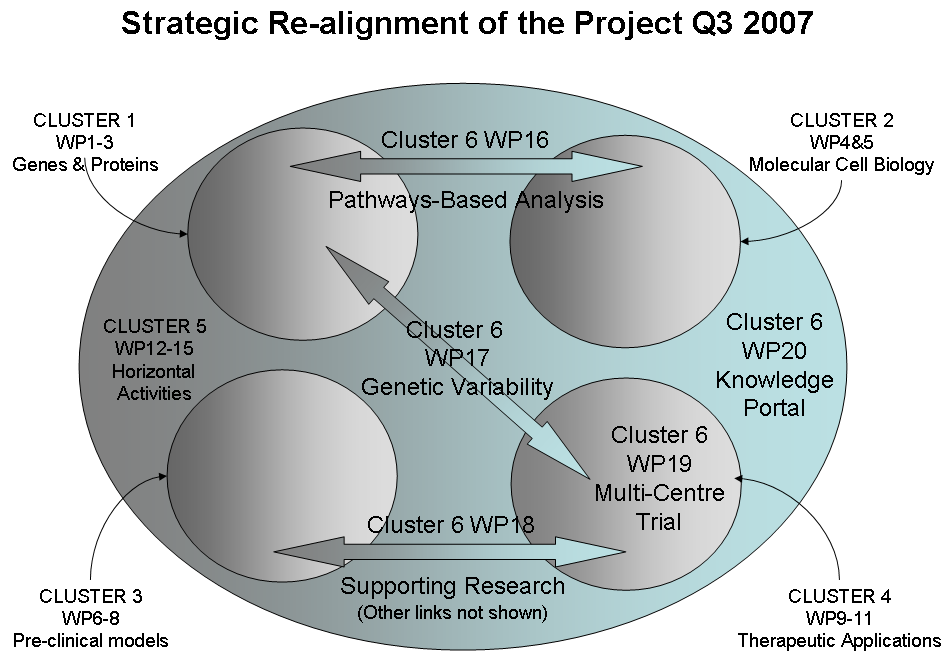
The above initiatives were further extended, outside of the Network, through links that were established with other FP6 projects, and with national or international societies, in the field or immunology or related areas. For example, DC-THERA promoted its activities, and made its first call for Associated Partners/Third Parties, at the International Dendritic Cell Meeting, ‘DC2006’, in Edinburgh GB. Over its lifetime, DC-THERA has also interacted closely with an FP6 Integrated Project on DC immunobiology (DC-VACC), and with NoEs on cancer immuntherapy (CIMT), cell migration and chronic inflammation (MAIN), and tissue engineering and regenerative medicine (ExperTissues). These interactions have included joint meetings (e.g. with DC-VACC), joint training courses (e.g. with MAIN), and strategy meetings (e.g. with CIMT in relation to the multi-centre clinical trial; see WP18). In the second half of the project the Network further developed a close interaction with the European Macrophage and Dendritic Cell Society (EMDS). For example at its 2007 meeting in Innsbruck AU, DC-THERA presented a workshop on ‘state-of-the-art imaging techniques’, and at its 2009 meeting in Regensburg DE the Network held a session on systems biology of DC. In addition, a joint nomenclature workshop was held at the 2008 meeting in Brescia IT, culminating in a joint report, made under the auspices of the WHO and the IUIS, and now published in the journal *Blood*.

#### Work package 15. Network management

Finally, it was essential to put in place a robust management structure to oversee the Network’s Joint Programme of Activities across all Clusters, to make strategic decisions with regard to these, and to implement and monitor the progress of the different Work Packages. This was the focus of WP15. For example DC-THERA Annual Meetings, and meetings of its Instutional Board and Executive Committee, took place in May 2005 in Venice IT and then, from 2007-2010 respectively, in Sardinia IT, Athens GR, Marseilles FR and again in Athens GR. [In 2006 the Annual meeting was devolved instead to a series of ‘Cluster meetings’ (relating to clusters 1-4)]. The later Annual Meetings were structured in sessions focussing on the four main areas of the S&T clusters, with increasing focus on areas relating to the activities in Cluster 6 (below); approximately 70 people attended each meeting, and at least two-thirds of all the partners and a number of Associated Partners/Third Parties were present at each. Throughout the project members of the Executive Committee also met in person on average at least three times a year, in addition to many regular conference calls between them. In the second half of the project in particular there were also a number of strategy meetings been those participants involved in the multi-centre clinical trial (WP18), and several workshops and training sessions in relation to the pathways-based initiative in WP16, noted above, and the knowledge portal in WP20. Several meetings were also held between the Project Coordinator and the Head of Unit and/or Project Officer at the European Commission; the latter also attended the later annual Network meetings. Overall this work package enabled the Joint Programme of Activities to be reviewed and revised, and the budget allocations to be adjusted in accordance with the Network’s priorities, and ensured that almost all its deliverables and milestones were achieved by the end of the project.

### Cluster 6. Strategic Priorities

An internal strategic review of the project was undertaken at its mid-point. This highlighted the many achievements that had been made, but it became clear that further integration of different key areas of the project were still needed, and that by identifying several new strategic priorities it would be possible to promote further collaborations between the Network’s participants. The budget was significantly adjusted and refocused to provide for these new initiatives, whilst still enabling others to continue such as the PhD studentships which were in progress, the Graduate Schools and the Annual Network Meetings. A total of five new Work Packages were created, that crossed existing Clusters and which are illustrated below.



#### Work package 16. Pathways-based analysis

The intracellular signalling pathways of DC can be viewed as a series of intersecting highways and byways leading from sensing by receptors at the plasma membrane, in intracellular compartments or the cytosol, to outcomes such as signalling to the nucleus for new gene expression, or changes in the cytoskeleton or cellular metabolism. Even though other cells may express any given receptor, the outcomes can be very different depending on the cell type in question. Bioinformatic tools can help to make biological sense of the vast number of changes in gene and protein expression that occur in response to any given stimulus [see cluster 1]. However this type of analysis is typically based on generic information in publicly-available databases such as KEGG that cannot be applied in a cell type-specific or dynamic (time-dependent) manner. A new approach was needed to identify and interrogate DC-specific signalling pathways. This was the focus of WP16, which complements and enhances the activities of Cluster 1 (WP1-3).

The DC-ATLAS initiative has developed a ‘road map’ of many of the key intracellular signalling pathways that are specifically operative in defined subsets of DC, in both humans and mouse. This comprises a database of validated DC-specific signalling pathways, DC-PATH, and a repository of microarrays (and some proteomic) experiments, DC-BASE, linked by new bioinformatic tools that were developed within the consortium (Florence, IT). DC-PATH was established after curation of an extensive number of pathways by experts in the field, including 14 of the Network’s participants. This database contains complete signalling pathways from Toll-like receptors TLR1-9 and C-type lectins such as DC-SIGN and dectin-1, as well as other pathways involved in central functions such as antigen processing and presentation. The curation process led to the potential exclusion of a large number of components that were not proven to exist in DC, and the discovery of >200 new components in the TLR pathways alone, including many new outcomes that had not before been documented.

DC-BASE now contains data from >250 individual microarray experiments, uploaded from the public domain (after quality control ‘cleansing’) or newly-generated within the consortium (cf. WP17, WP18). The newly-developed bioinformatic tools enable both pathways-based analysis of microarray data (by linking DC-BASE with DC-PATH) and different microarray experiments to be compared to enable ‘pathway signatures’ to be identified. DC-ATLAS is the very first initiative fully to comply with the latest systems biology (SBGN) standards, it enables pathways analysis to be carried out simultaneously on both genomic and proteomic (and potentially other ‘-omics’) data, and is underpinning other initiatives such as retrospective analysis of patient samples as in WP17 (below). General access to these resources, and the new bioinfomatic tools, will be made available by end 2010.

#### Work package 17. Retrospective patient profiling

Many of the Network participants involved in clinical trials of DC-based vaccination, particularly for cancer, have large repositories (‘biobanks’) of frozen patient cells. These include peripheral blood mononuclear cells (PBMC), monocytes (used to generate DC), and DC before and after maturation for vaccine generation. In some cases, these DC preparations have been generated using standardised protocols and techniques, thus permitting comparisons to be readily made between them. Potentially, retrospective transcriptional profiling and pathways-based analysis of these (cf. WP1, WP16) could reveal ‘signatures’ that are associated with outcome, i.e. immunological or clinical responses This is the focus of WP17. Before this work could commence it was necessary to carry out a number of pilot experiments to ensure that samples were adequate for this type of analysis (e.g. that mRNA was of a suitable quality, since samples were frozen in different ways). It was also necessary to compare samples processed for the ‘Affymetrix’ and ‘Illumina’ platforms to ensure that pathways data were comparable. In addition, new experiments had to be performed to obtain ‘reference datasets’ for both this work and that in WP16, for example to generate transcriptional data from standardised DC preparations exposed to defined maturation stimuli.

Two centres, in Erlangen DC and Nijmegen NL, are now progressing the main studies in this work package. Between them, ~100 melanoma patients have been identified who were treated with DC vaccination and from whom corresponding samples of DC vaccines and cells such as PBMC are available. These have been divided into 3 cohorts based on whether or not the vaccine induced immunological responses and whether or not the patients lived for >24 months after treatment [no patient lived >24 months unless an immunological response was detectable]. Comparable DC vaccine samples have been identified for each patient, together with corresponding PBMC to act as a ‘baseline’. These samples are currently being processed for ‘Illumina’ transcriptional profiling, after which the data are being uploaded into the DC-BASE repository. Pathways-based analysis of these data (as in WP16) should be completed by end 2010. If successful this initiative may, for the first time, identify pathway signatures that correlate with different types of immunological response and/or a better clinical outcome. This information could then be used rationally to design more effective DC vaccines for the future.

#### Work package 18. Collaborative research projects

To enhance the work within Cluster 6, a call for proposals for small, short-term (e.g. maximum of € 40K over12 months), collaborative research projects was made in any area relevant to other Work Packages of the Cluster. This was the focus of WP18. After anonymised review of each by three independent experts, according to strict selection criteria, 12 projects were selected for funding, involving 10 Partners and 5 Associated Partners/Third Parties. About half of these projects were relevant to WP16 and have provided datasets to enhance the DC-BASE repository and the remainder were for pre-clinical or clinical studies relevant to WP18 or other areas of the project which were continuing.

#### Work package 19. Multi-centre clinical trial

A large number of clinical trials of DC-based vaccination for cancer have been and are being performed by both Network participants and by many others internationally. Europe now leads this field, with perhaps the two most promising of the currently available approaches having been developed by Partners of DC-THERA: DC-based vaccination strategies using ‘TRI-MIX’ mRNA, or vaccination with long peptides and adjuvant (Cluster 3, WP8; Cluster 4, WP9). A major problem has, however, been the lack of standardisation across trials conducted to date, both in terms of the DC vaccines and vaccination schedules that are used, and the immunomonitoring techniques that are employed. This largely prevents meaningful comparisons to be made between the outcomes of different trials, and reduces the overall value of the information that is gained for the future design of new strategies. To overcome these limitations, standardised multi-centre clinical trials are needed. This is the focus of WP19.

The original objective of this work package was to conduct, within DC-THERA, the first well-standardised, two-armed, multi-centre clinical trial ever to be performed – across three centres in Brussels BE, Erlangen DE and Nijmegen NL – using consensus SOPs that were drawn up in each of the participating centres. The original aim of the trial was to assess immune responses of melanoma patients treated with either long peptides plus adjuvant, or with DC-based immunotherapy (cf. WP9). However, at the last moment, the company supplying the peptides withdrew the licence for clinical use of these peptides and, despite an intensive search, the Network was unable to identify an alternative supplier.

After further intensive discussions between the participants, the trial was then restructured such that each centre would compare DC transfected with mRNA coding for constitutively active TLR-4, CD40 and CD70 (‘TRI-MIX’; see WP7) as a maturation stimulus, as an additional arm (10 patients each) to the different DC-based strategies that were already in clinical trials at each of the respective centres for treatment of advanced melanoma patients. Hence, collectively, the partners are now comparing up to 8 different modalities using DC matured with the ‘standard’ cytokine cocktail versus TRI-MIX, delivered intravenously, intradermally or intranodally and which have been co-transfected with mRNA encoding different tumour antigens.

It was further agreed, to enable comparison between outcomes, that standardised immunomonitoring procedures would also be adopted by the three centres involved. This necessitated, for example, revision of SOPs relating both to DC generation and monitoring, an application from one of the centres for a licence to produce GMP-grade RNA for all three (since no other suitable supplier could be identified in Europe or elsewhere), and compliance with both national and European regulations. All this has now been achieved over two-thirds of the patients had been vaccinated. This work is now on schedule for completion of vaccination and initial immunomonitoring of 30 patients by the end of 2010.

#### Work package 20. Knowledge portal

A final objective of DC-THERA was to enhance the sharing of information relating to the expertise and resources of each Network participant, and potentially to incorporate additional, similar information from other scientists, clinicians and SMEs in this or related areas. This was the focus of WP20. The Network has now developed a database of the existing expertise and resources of all Partners and Associated Partners/Third Parties within the Network, the DC-THERA Knowledge Portal (also termed the DC-THERA Directory). Such expertise includes data sets, protocols, tools, and bio-materials of the persons and participating companies within the DC-THERA Network. This project was initially intended to enhance collaboration within the network and to serve as a reference. To enhance collaboration, it provides multiple ways to access the information, through queries and through directories. For each entry in the database it provides links to relevant expertise and partners within the network. For its development, we have opted for a web interface and an early-prototype approach. The semantic organization, including browsing and querying, of the directory is based on fragments of standard ontologies, in particular OBI and terminologies developed within DC-ATLAS (see WP16), thus providing a seamless interface between the two. The first release of the DC-THERA directory was made in mid-2008 (based on the information and ontologies from past Annual Reports). Following this, a panel of reviewers was assembled to provide feedback, and a second release was made towards the end of 2009. This initiative – which has potentially integrated the work of all WPs of the Project – will be made generally accessible by the end of 2010.

## 2. Dissemination and use

Throughout this project a number of Partners (and Associated Partners/Third Parties) have produced exploitable knowledge, particularly in the form of patents relating to various discoveries. The respective partners have, or will have, taken the appropriate measures to protect their own Intellectual Property Rights. However the Project Coordinator has not been informed of any exploitable knowledge arising from collaborative work by Network participants. The principal outputs of the collaborative work within this project, which cannot readily be defined as ‘exploitable knowledge’ although they are contribute to future ‘dissemination and use’ are primarily the following.

1. The DC-ATLAS initiative [WP16]
   1. The DC-PATH database of DC-specific pathways
   2. The DC-BASE database of transcriptional profiling experiments
   3. The DC-STUDIO bioinformatic tools
2. The DC-THERA Knowledge Portal [WP20]
3. The clinical trials database [WP9]

All will be made accessible, either open access or on a subscription basis, by end 2010.

***A list of all DC-THERA Partners, Associated Partners and Third Parites follows.***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Principal Partners** | | | | | | |
| **Partner** | **Lab Head Name/Scientific contact** | | **Partner Organisation** | | **Country** | |
| **1** | **COORDINATOR Jonathan Austyn Gordon G MacPherson, Vincenzo Cerundolo** | | **University of Oxford** | | **UK** | |
| **2** | **Carl G. Figdor, Gosse Adema** | | **Stichting Katholieke Universiteit University Medical Centre Nijmegen** | | **NL** | |
| **3** | **Muriel Moser** | | **Universite Libre de Bruxelles** | | **BE** | |
| **4** | **Anne O'Garra** | | **Medical Research Council** | | **UK** | |
| **5** | **Francesca Granucci** | | **University of Milano-Bicocca** | | **IT** | |
| **6** | **Gerold Schuler,  Alexander Steinkasserer** | | **Friedrich Alexander Universitat Erlangen-Nurnberg** | | **DE** | |
| **7** | **Robert Coffin** | | **Biovex** | | **UK** | |
| **8** | **Catherine.De.Greef** | | **Brucells** | | **BE** | |
| **9** | **Ugo D'Oro** | | **Chiron** | | **IT** | |
| **10** | **Andrea Splendiani** | | **Leaf Biosciences** | | **IT** | |
| **11** | **Charles Nicolette** | | **Argos Therapeutics, Inc** | | **DE/USA** | |
| **12** | **Filippo Petralia** | | **SEKMED** | | **IT** | |
| **13** | **Sebastian Amigorena** | | **Institut Curie** | | **FR** | |
| **14** | **Thierry Boon, Pierre Coulie** | | **Christian de Duve Institute** | | **BE** | |
| **15** | **Duccio Cavalieri** | | **University of Florence** | | **IT** | |
| **16** | **Sandra Gessani** | | **Istituto Superiore di Sanità** | | **IT** | |
| **17** | **Nicolas Glaichenhaus** | | **Université de Nice-Sophia Antipolis Institut Universitaire de France** | | **FR** | |
| **18** | **Rolf Kiessling, Pavel Pisa, Hakan Mellstedt** | | **Karolinska Institutet** | | **SE** | |
| **19** | **Relocated - now Partner 33** | | | |  | |
| **20** | **Relocated - now Partner 34** | | | |  | |
| **21** | **C.J.M. Kees Melief** | | **Leiden University Medical Center** | | **NL** | |
| **22** | **Philippe Pierre** | | **Centre d'Immunologie de Marseille-Luminy CNRS-INSERM-Univ. Med.** | | **FR** | |
| **23** | **Maria Pia Protti** | | **Scientific Instituto San Rafaelle** | | **IT** | |
| **24** | **Alexander Scheffold, Andreas Radbruch,Andreas Thiel** | | **Deuttsches Rheuma-Forschungszentrum Berlin** | | **DE** | |
| **25** | **Caetano Reis e Sousa** | | **Cancer Research UK** | | **UK** | |
| **26** | **Benedita Rocha** | | **INSERM U591 Institut Necker** | | **FR** | |
| **27** | **Federica Sallusto, Antonio Lanzavechia, Markus Manz, Mariagrazia Uguccioni** | | **Institute for Research in Biomedicine, Bellizona** | | **CH** | |
| **28** | **Mark Suter** | | **University of Zurich** | | **CH** | |
| **29** | **Kris Thielemans** | | **Vrije Universiteit Brussel** | | **BE** | |
| **30** | **Hermann Wagner** | | **Technische Universität München** | | **DE** | |
| **31** | **Laurence Zitvogel** | | **Institut Gustave Roussy** | | **FR** | |
| **32** | **Paola Ricciardi - Castagnoli** | | **GENOPOLIS** | | **IT** | |
| **33** | **Matthias Mann** | | **Max-Planck-Institut für Biochemie** | | **DE** | |
| **34** | **Alberto Mantovani** | | **Istituto Clinico Humanitas** | | **IT** | |
| **Associated Partners** | | | | | | | |
| **Associated Partner** | | **Lab Head Name/Scientific contact** | | **Partner Organisation** | | **Country** | |
| **101** | | **Benny Chain** | | **Windeyer Institute of Medical Sciences/University College London** | | **UK** | |
| **102** | | **Frank Nestle** | | **King's College London School of Medicine** | | **UK** | |
| **103** | | **INSERM U601/Nancy** | | **INSERM U601/Nancy** | | **FR** | |
| **104** | | **Regis Josien** | | **INSERM U643/Nantes** | | **FR** | |
| **105** | | **Jacques Bartholeyns** | | **IDM/SME in Paris** | | **FR** | |
| **106** | | **Annika Scheynius** | | **Karolinska University Hospital/Stockholm** | | **SE** | |
| **107** | | **Maria Rescigno** | | **European Institute of Oncology/Milano** | | **IT** | |
| **108** | | **Ignacio Melero** | | **University of Navarra/Pamplona** | | **ES** | |
| **109** | | **Rik Scheper** | | **VU University Medical Center/Amsterdam** | | **NL** | |
| **110** | | **Éva Rajnavölgyi** | | **University of Debrecen/Debrecen** | | **HU** | |
| **111** | | **Suzanne Watts** | | **National Blood Service** | | **UK** | |
| **112** | | **Luis Graca** | | **Universidade de Lisboa Faculdade de Medicina** | | **PT** | |
| **113** | | **Juri Rappsilber** | | **Wellcome Trust Centre for Cell Biology University of Edinburgh** | | **UK** | |
| **114** | | **Edwin Lasonder** | | **University of Nijmegen** | | **NL** | |
| **115** | | **Janet Fernihough** | | **Ribostem** | | **UK** | |
| **116** | | **Meredith O'Keefe** | | **Bavarian-Nordic** | | **DE** | |
| **117** | | **Walter Reith** | | **University of Geneva** | | **CH** | |
| **118** | | **Jirina Bartunková** | | **Institute of Immunology 2nd Medical School, Charles University** | | **CZ** | |
| **119** | | **Mai-Britt Zocca** | | **Dandrit Biotech A/S** | | **DK** | |
| **120** | | **Thomas Felzmann** | | **CCRI-Austria** | | **AT** | |
| **121** | | **Thomas Brocker** | | **Ludwig-Maximilians-Universität München** | | **DE** | |
| **122** | | **Mark Aloysius** | | **Nottingham University** | | **UK** | |
| **123** | | **Elena Ranieri** | | **University of Foggia** | | **IT** | |
| **124** | | **A. A. van de Loosdrecht** | | **VU Medical Center/Amsterdam** | | **NL** | |
| **125** | | **Henri de la Salle** | | **INSERM U725** | | **FR** | |
| **127** | | **Guido Vanham** | | **Inst of Tropical Medicine, Antwerp.** | | **BE** | |
| **128** | | **Inge Marie Svane/Ozcan Met** | | **Herlev University Hospital** | | **DK** | |
| **129** | | **Gerard Bos** | | **Academic Hospital,Maastricht** | | **NL** | |
| **130** | | **Silvia della Bella** | | **University of Milan** | | **IT** | |
| **131** | | **Luis Ferreira Moita** | | **Inst. Molecular Medicine/University Lisbon** | | **PT** | |
| **132** | | **Tina Zavasnik Bergant** | | **University in Slovenia** | | **SI** | |
| **133** | | **Miodrag Colic** | | **Institute of Medical Research** | | **YU** | |
| **134** | | **Casamassima Addolorata** | | **IRCCS-Oncology/Bari** | | **IT** | |
| **135** | | **Ihsan Gursel** | | **Molecular Biology Dept/ Bilkent University** | | **TR** | |
| **136** | | **S. Jung** | | **Weizmann Institute** | | **IL** | |
| **137** | | **Manfred Lutz** | | **University of Wuerzburg** | | **DE** | |
| **138** | | **Shalin Naik** | | **The Netherlands Cancer Institute** | | **NL** | |
| **139** | | **Sorin Draghici** | | **Wayne State University, Michigan** | | **US** | |
| **141** | | **Shoshanna Frankenburg** | | **Hadassah Medical Organization** | | **IL** | |
| **142** | | **Tim Sparwasser** | | **University of Hannover** | | **DE** | |
| **143** | | **Bart Lambrecht** | | **University of Ghent** | | **BE** | |

|  |  |  |  |
| --- | --- | --- | --- |
| **Third Parties** | | | |
| **Third Party** | **Lab Head Name/Scientific contact** | **Partner Organisation** | **Country** |
| **301** | **Benny Chain** | **Windetyer Institute of Medical Sciences, University College London** | **UK** |
| **302** | **Frank Nestle** | **Kings College School of Medicine** | **UK** |
| **303** | **Marc Gregoire** | **INSERM U601/Nancy** | **FR** |
| **304** | **Regis Josien** | **INSERM U643/Nantes** | **FR** |
| **305** | **Annika Scheynius** | **Karolinska University Hospital, Stockholm** | **SE** |
| **306** | **Maria Rescigno** | **European Institute of Oncology, Milano** | **IT** |
| **307** | **Ignacio Melero** | **University of Navarra, Pamplona** | **ES** |
| **308** | **Rik Scheper** | **VU University Medical Centre** | **NL** |
| **309** | **Eva Rajnavolgyi** | **University of Debrecen, Debrecen** | **HU** |
| **310** | **Suzanne Watts** | **National Blood Service** | **UK** |
| **311** | **Luis Graca** | **Universidade de Lisboa, Faculdade de Medicina** | **PT** |
| **312** | **Edwin Lasonder** | **University of Nijmegen** | **NL** |
| **313** | **Janet Fernihough** | **Ribostem** | **UK** |
| **314** | **Meredith O'Keefe** | **Bavarian-Nordic** | **DE** |
| **315** | **Jirina Bartunkova** | **Institute of Immunology, 2nd Medical School, Charles University** | **CZ** |
| **316** | **Mai-Britt Zocca** | **Dandrit Biotech A/S** | **DK** |
| **317** | **Thomas Felzmann** | **CCRI Austria** | **AT** |
| **318** | **Elena Ranieri** | **University of Foggia** | **IT** |
| **319** | **Henri de la Salle** | **INSERM U725** | **FR** |
| **320** | **Guido Vanham** | **Institute of Tropical Medicine, Antwerp** | **BE** |
| **321** | **Inge Marie Svane** | **Herlev University Hospital** | **DK** |
| **322** | **Bart Lambrecht** | **University of Ghent** | **BE** |
| **323** | **Tina Zavasnik Bergant** | **University in Slovenia** | **SI** |
| **324** | **Miodrag Colic** | **Institute of Medical Research** | **YU** |
| **325** | **Ihsan Gursel** | **Molecular Biology Department, Bilkent University** | **TR** |
| **326** | **Steffen Jung** | **Weizmann Institute** | **IL** |
| **327** | **Manfred Lutz** | **University of Wuerzberg** | **DE** |
| **328** | **Shalin Naik** | **The Netherlands Cancer Institute** | **NL** |
| **329** | **Shoshanna Frankenberg** | **Hadassah Medical Organisation** | **IL** |

# Section 2

# Final Plan for using and disseminating the knowledge

Examples of **publishable results of the PUDK** are given in the ‘[Individual Partner Contributions](#_Work_package_11.)’ (scientific report) within the Periodic Activity Report, at the end of Section 2.

Examples of **dissemination of knowledge from the Network** to outside of it (e.g. to a large number of other FP6 Projects, as well as Networks, International Societies and a European Regulatory Body) are given in the Periodic Activity Report, Section 3, Cluster 5, Work Package 14. Other examples of (e.g. international conferences) are given in [‘DC-THERA Dissemination Activities 2009-2010’](../Periodic%20Report/Whole%20Report/LINKS/Dissemination%20Activities%202010.docx)

You can also find details of all of DC-THERA’s strategy, planning and dissemination events in the ‘[Status Chart’](../Periodic%20Report/Whole%20Report/LINKS/DC-THERA%20Status%20Chart%202009-10.xls).

Examples of **dissemination of knowledge within the Network**, between Partners and Associated Partners/Third Parties, are given in ‘[DC-THERA Research Integration](../Periodic%20Report/Whole%20Report/LINKS/Integration%20Activity%20from%20Science%20reports.docx)’ subsection within the Periodic Activity Report at the end of Section 3.

The table below gives examples of output from the Network since it began, to the end of the current period

| **Planned / actual dates** | **Type** | **Type of audience** | **Countries addressed** | **Size of audience** | **Partner responsible / involved** |
| --- | --- | --- | --- | --- | --- |
| August 2005 | DC-THERA Website (ScienceDev, Milano) | All | Global | ~100,000 | \*P1 |
| October 2005 | Press release re DC-THERA: University of Oxford GB | Higher education | UK and global | ~25,000 | P1 |
| Article re DC-THERA: University of Oxford newsletter ‘Blueprint’ | Higher education | UK | ~15,000 | P1 |
| Newspaper articles re DC-THERA: Oxford Times & Oxford Mail | General public | UK | ~50,000 | P1 |
| November 2005 | Press release: Christian de Duve Institute Bruxelles, BE | Higher education | BE and global | ~25,000 | P14 |
| November 2005 | Interview for e-journal ‘Revista Fapesp’’ Brazil, South America | General public | Brazil, South America | 150,000 | P1 |
| June 2006 | Joint meeting with FP6 IP DC-VACC | Higher education | IT& Pan-European | 50 | P1, P5 |
| September 2006 | Conference: gold sponsor of ‘DC2006’, Edinburgh UK | Higher education, research, clinical, industrial (Pharma) | UK and global | 1,000 | \*P1 |
| Exhibition: stand and promotional materials at ‘DC2006’ |
| Poster: ‘DC2006’ stand |
| Flyers: ‘DC2006’ delegate bags |
| December 2006 | DC-THERA website (NetSight UK) | All | Global | ~100,000 | \*P1 |
| January 2007 | Meeting with the European Medicines Agency (EMEA) | EMEA Regulators/ Scientific Board Cell Therapies | Pan-European Panel | ~40 | P06 |
| March 2007 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 30 | \*P1 |
| July 2007 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 30 | \*P1 |
| July 2007 | DC2007 DC Vaccination Meeting, Bamberg DE | Higher education, research, clinical, industrial (Pharma) | Pan-European | ~300 | P06 |
| August 2007 | DC-THERA at ‘ImmunoRio’; Brazil, South America | Higher education, research, clinical, industrial (Pharma) | Global | ~100 | P01 |
| September 2007 | DC-THERA & European Macrophage and Dendritic cell Society (EMDS) Joint Meeting, Innsbruck AT | Higher education, research, clinical, industrial (Pharma) | Global | ~300 | P02 |
| September 2007 | Article on DC-THERA ‘A Arte do Encontro’ by Carlos Fioravante in *Pesquisa Fapesp*; Brazil, South America | General Public | Brazil & South America | ~150,000 | \*P1 |
| October 2007 | Speaker at EMEA meeting on guidelines in Cell-Based Therapies, Lisbon PT | Regulators,Higher education, research, clinical, industrial (Pharma) | Pan-European | ~250 | P06 |
| November 2007 | EMEA meeting with ‘ExperTissues’ NoE, Brussels BE | Regulators,Higher education, research, clinical, industrial (Pharma) | Pan-European | ~250 | P01 |
| December 2007 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 30 | \*P1 |
| April 2008 | DC-THERA Participation in Portuguese Stem Cells Meeting, Faro PT | Higher education, research, clinical, industrial (Pharma) | Global | ~300 | \*P01 & external |
| March 2008 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 30 | \*P1 |
| May 2008 | Workshop on NoE sustainability, Brussels BE | Higher education, research, clinical, industrial (Pharma) | Global | ~300 | \*P01 & EC |
| July 2008 | Joint workshop with ‘MAIN’ NoE, Nijmegen NL | Higher education, research, clinical, industrial (Pharma) | Pan-European | 100 | P2 |
| July 2008 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 50 | \*P1 |
| September 2008 | DC-THERA & European Macrophage and Dendritic cell Society (EMDS) Joint Meeting, Brescia IT & DC-THERA (WHO / IUIS) workshop on DC nomenclature | Higher education, research, clinical, industrial (Pharma) | Global | ~300 | P02 |
| 15 December 2008 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 70 | \*P1 |
| April 2009 | ECPMA Association of Project Managers International Meeting | FP6 Project Managers and coordinators | Pan-European | 70 | P1 |
| March 2009 | DC-CREST Graduate School for Students held in Celerina Switzerland | Higher education, research, clinical, industrial (Pharma) | Global | 40 | P28\*, P01\* and other members of the network (Partners and Associated Partners/ Third Parties) |
| 6th to 10th May 2009 | DC-THERA Annual Meeting in Marseilles | Higher education, research, clinical, industrial (Pharma) | Pan-European | 76 | P01\* and other members of the network  (Partners and Associated Partners/ Third Parties) |
| September 2009 | European Macrophage and Dendritic cell Society (EMDS) Meeting with DC-THERA sponsored Session, Regensberg DE | Higher education, research, clinical, industrial (Pharma) | Global | ~300 | P01\*, P02, P05, P15, 3rd Parties |
| March 2010 | DC-CREST Graduate School for Students held in Celerina Switzerland | Higher education, research, clinical, industrial (Pharma) | Global | 40 | P28\*, P01\* and other members of the network (Partners and Associated Partners/ Third Parties) |
| 5th to 9th May 2010 | DC-THERA Final Annual Meeting in Athens | Higher education, research, clinical, industrial (Pharma) | Pan-European | 76 | P01\* and other members of the network  (Partners and Associated Partners/ Third Parties) |
| May 2010 | Gordon Conference in Immunochemistry and Immunobiology 2010 – DC-THERA Sponsored  Cancer Research UK | Higher education, research, clinical, industrial (Pharma) | Global | ~300 | P25\* and other members of the network |
| End 2010 | Clinical Trials Database | Higher education, research, clinical, industrial (Pharma) | Global | ~10,000 | P06 |
| End 2010 | DC-ATLAS | Higher education, research, clinical, industrial (Pharma) | Global | ~10,000 | P15\* |
| End 2010 | DC-THERA Knowledge Portal | Higher education, research, clinical, industrial (Pharma) | Global | ~100,000 | P10 |

\* On behalf of the DC-THERA Executive CommitteeSection 3

# Final Management Report

## Final Summary Financial report

[This information is provided in TABLE C: DC-THERA Summary Financial Report](../Periodic%20Report/Whole%20Report/LINKS/TABLE%20C%20DC-THERA%202005%20-%202010%20Summary%20Financial%20Report.xls)

# Final Report on the distribution of the Communities Contribution

## Final Report on the distribution of the communities contribution

[This information is provided in TABLE D: DC-THERA Distribution of Contribution](../Periodic%20Report/Whole%20Report/LINKS/TABLE%20D%20DC-THERA%202005%20-%202010%20Distribution%20of%20Contribution.xls)