Executive summary:

The EuroSyStem Project is concerned with increasing our understanding of stem cells and how they function to maintain and repair tissues. The project aimed to develop deeper scientific knowledge, new technological platforms, advanced computational tools and improved information resources for stem cell biology. In addition EuroSyStem sought to act as a federating force for European researchers working in the field of fundamental stem cell biology.

The project identified a group of major goals, each of which has been substantially achieved over the four years:
- New knowledge and understanding of the cellular organisation and complexity of the major mammalian stem cell systems
- Delineation of generic and specific features of molecular networks that govern self-renewal, commitment and potency in different stem cell systems
- Refined methodologies for live cell imaging and isolation, and for quantitative molecular interrogation of stem cells at both population and single cell levels
- Creation of computational tools and database resources to enable and facilitate systems approaches to stem cell biology
- Delivery of a range of training activities to engage computational and mathematical biologists with stem cell research, to disseminate specialist skills, and to spread theoretical and practical knowledge
- Federation of key investigators to bring consolidation, cohesion and competitiveness in fundamental stem cell research across Europe
- Provision of accessible information and outreach for engaging the wider scientific community and lay public in dialogue about stem cell biology

The research results are available in 128 publications (including two in press) in international peer-reviewed journals and datasets are deposited in relevant public databases including the custom-designed StemDB (see http://www.stemdb.org/ online). Information is provided in accessible form to a wider public through the EuroStemCell portal (see http://www.eurostemcell.org online) that EuroSyStem fostered.

Finally, an over-arching ambition of the EuroSyStem collaborative project has been to integrate the high quality but dispersed capabilities of European stem cell research into a cohesive network. The consolidated framework for internationally competitive research collaboration established by EuroSyStem provides an enhanced environment for training, retention and recruitment of leading investigators. This is essential to ensure that Europe remains at the forefront of global stem cell discovery and acquires the knowledge to enable successful biopharmaceutical and medical applications in future.

Project Context and Objectives:

The EuroSyStem Project brought together elite European research teams to create a unique and world leading programme in fundamental stem cell biology. The research teams were drawn from both the experimental and computational communities. The ambition was to interlink these two areas in an integrated approach and thereby to drive the generation of new knowledge on the characteristics of normal and abnormal stem cells. We concentrated on illuminating the molecular circuitry governing the decision between self-renewal and differentiation. Our primary focus has been on the paradigmatic mammalian stem cell systems; haematopoiesis and epithelia in vivo, embryonic and neural stem cells in vitro. Selected analyses and screens in genetically amenable model organisms have enriched particular areas of investigation. By measuring and modelling stem cell properties and behaviour we provide radically new information on interacting networks of genes and proteins, paving the way for advanced systems biology approaches.

In a series of focussed collaborative workpackages EuroSyStem investigator explored issues of cellular hierarchy, signalling, epigenetics, dysregulation, and plasticity, within and between systems. In a further mechanism-centred workpackage, the connections between asymmetric division, signalling cross-talk, transcriptional circuitry, and the niche were examined for different stem cells. The overall multidisciplinary approach included transgenesis, real time imaging, multi-parameter flow cytometry, transcriptomics, RNA interference, proteomics and single cell methodologies, with bioinformatic and computational approaches integrated throughout. small and medium-sized entreprises (SME)s contributed to the development of enhanced resolution and quantitative technologies. A platform workpackage provided new computational tools and database resources, enabling implementation of novel analytical and modelling approaches. A scientific advisory panel of leading experts in systems biology reviews advised the project at each annual review.

EUROSYSTEM also aimed to engaged widely with, and provide a focal point for, the European stem cell research community, for example through organising high-level European stem cell conferences with sponsorship from EMBO. A network of excellent independent investigators was envisioned, embracing stem cell researchers working in different tissues and organisms. This network will promote exchange, interaction and synergy, accelerating progress to a deeper and more comprehensive understanding of stem cell properties. In order to foster a cohesive and flourishing basic stem cell research sector EUROSYSTEM devoted resources to arranging annual symposia, training workshops, summer schools, networking and collaborative research opportunities. In parallel EUROSYSTEM has sought to develop a range of WEB resources, educational and outreach materials for the wider community of scientists, clinicians, patient groups, regulators, and lay public.

EUROSYSTEM set and achieved the following major objectives over the four years of the project:
- To acquire new knowledge and understanding of the cellular organisation and complexity of the major mammalian stem cell systems
- To delineate the generic and specific features of networks that govern self-renewal, commitment and potency in different stem cell systems

- To develop refined methodologies for cell imaging and isolation, and for quantitative molecular interrogation of stem cells at both population and single cell levels
- To generate computational tools and database resources that will enable and facilitate systems approaches to stem cell biology
- To implement a range of training activities that will engage computational and mathematical biologists with stem cell research, disseminate specialist skills and spread theoretical and practical knowledge
- To achieve consolidation, cohesion and competitiveness in fundamental stem cell research in Europe through creation of a federation of EUROSYSTEM partners with 40 Associate Principal investigators constituting a virtual European Centre of Excellence
- To provide accessible information and outreach for engaging both the wider scientific communities and lay public in dialogue about stem cell biology

Project Results:
The consortium aimed to maximize cross-fertilization and synergy between the participating research teams, and in particular strove to foster a new interface between experimentalists and computational scientists. This was considered essential to maximize integration of rapidly accumulating global genomics and proteomics data into the project. The research plan was constructed wherever feasible around collaborative projects rather than individual contributions. The project design provided a flexible structure in which individuals could readily move between work packages. Furthermore, in order to stimulate and enable new directions and interactions, a reserve fund was set aside specifically to support innovative sub-projects proposed jointly by Principal Investigators. This strategy has proven extremely valuable by encouraging fresh collaborations which emerged organically as the project evolved.

The work plan was structured into four fundamental research work packages, each comprised of several interconnected sub-projects, plus two platform work packages. Work packages 1 to 3 centred on the respective major stem cell categories under investigation; haematopoietic, epithelial, and cultivated stem cell lines. This structure allows for the distinctive biology of each system and assembles critical mass for a coherent focus on fundamental characterization and analysis. Importantly, each of these work packages was organised around five key cross-cutting themes: cell hierarchy, signaling and fate choice, epigenetics, dysregulation, and plasticity & reprogramming. Work package 4 interlinked the different systems by addressing generic mechanistic issues, using the most appropriate stem cell model on a case by case basis. Work packages 5 and 6 provided platforms and resources to support both the consortium and the wider stem cell research community. Work package 5 was dedicated to data management, data analysis infrastructure and computational tools. Work package 6 comprised training, federating and outreach components.

a) Work Package 1: Towards a systems approach for haematopoietic stem cell differentiation

Haematopoietic stem cells are the best characterised somatic stem cell type and thus provide a good paradigm in which to generate datasets and mathematical tools aimed at beginning to develop a deep systems level understanding of self-renewal and differentiation decisions. We have exploited this system in order to gain insight into the system's level

architecture of cell fate choice. To achieve this end we have conducted research under the following 4 thematic areas:

Theme 1: Cellular hierarchy
To functionally define cell hierarchies within the murine HSC compartment using cell surface marker and genetically engineered strains of reporter mice.

Theme 2: Signalling and fate choice
Identification of candidate regulators of murine HSC function and their genome-wide transcriptional
network relationships with the aim to in silico integrate datasets and their subsequent use for network inference and dynamic modelling approaches.

Theme 3: Epigenetics
To test selected candidates focussing on epigenetic regulators, transcription factors, cytokine receptor and microenvironmental/niche related signalling and extending the analysis of key regulatory molecules into human cells.

Theme 4: Dysregulation and Plasticity and reprogramming
To perturb key self-renewal regulators and assess and mathematically model the effect on disruption of lineage commitment (increased plasticity) in stem cell daughters.

i) WP1 Objectives
- To functionally define cell hierarchies within the murine HSC compartment using cell surface marker and genetically engineered strains of reporter mice.
- Identification of candidate regulators of murine HSC function and their genome-wide transcriptional network relationships with the aim to in silico integrate datasets and their subsequent use for network inference and dynamic modelling approaches.
- To test selected candidates focussing on epigenetic regulators, transcription factors, cytokine receptor and microenvironmental / niche related signalling and extending the analysis of key regulatory molecules into human cells.
- To perturb key self-renewal regulators and assess and mathematically model the effect on disruption of lineage commitment (increased plasticity) in stem cell daughters.

ii) WP1 Results

Scientists within this work package have exploited their findings to make discoveries beyond those initially envisaged at the beginning of the project. An area of note in this respect has been the functional analysis of genes, particularly regulatory molecules, identified through a panoply of systems level discovery technologies.

A key goal at the outset of this enterprise was to gain insight into the topology, interaction logic and dynamic behaviour of transcription factor circuits that specify cell fate and regulate the blood system. Such a goal requires knowledge both of the cellular pathways and intermediates that function as checkpoints en route to lineage commitment and differentiation, and of the identity of key transcription factors or other regulatory molecules that function as instructive determinants of

cell fate choice. Significant data on both these aspects have been accumulated during the course of the project, providing the bases for considering how fate regulatory circuits may be wired. Modelling of such circuits predicts dynamic behaviour which subsequently may be tested experimentally.

In this regard we have established a series of principles governing the logic of interaction between key nodal regulators such as GATA-1, GATA-2 and PU.1 and extrinsic inputs such as those provided by cytokines involved in cell growth and lineage choice.

These first generation models have become more sophisticated as we have identified multiple cross interactions between transcriptional regulators on their own as well as each other's loci from genome wide as well as locus targeted ChIP-sequencing studies.

These models are predictive – thus searching parameter space for different circuit assemblies returns a limited number of solutions that afford gene expression characteristics compatible with experimental information. When tested such inferences have proven robust and open the way to incremental step-wise building of large circuits linking novel regulators into the core kernels we have established. This approach contrasts with global network inference attempts which have met with only limited success. We anticipate however that as additional links are added to the provisional subnetworks the possibilities remaining for as yet unlinked players will begin to become canalized and the two approaches will start to mesh in a manner assisted by our studies of cis-regulatory motifs, integration of genetical genomics and transcriptional data sets, and by our cell based models.

iii) WP1 Outputs

Relevant data has been deposited in public databases. Please see individual deliverable reports for full details of these database deposits.

This work package has resulted in the following outputs:
- 16 peer-reviewed research publications
- 5 reviews
- 0 patents
- 3 other products
- An additional 11 manuscripts are currently under review or in preparation

b) Work Package 2: In vivo analysis of molecular programs underlying normal and diseased epithelial stem cells

In this work package we aimed to identify and compare the cellular and molecular relationships between epithelial stem cells of different origin (squamous simple intestinal, thymic and glandular), and in disease states. Epithelial stem cells often reside in specialized microenvironments (niches), which are thought to be important for stem cell maintenance, proliferation and differentiation. We planned to dissect this relationship using genetically engineered reporter and conditional knockout mice. The specific goals were:

Theme 1: Cellular hierarchy and stem cell identity

Use of stem cell markers combined with genetically engineered reporter mice to isolate and monitor the differentiation potential and gene expression profile of epithelial stem cells and cancer stem cells of different origin.

Theme 2: Signalling and fate choice
Functional tests using gain and loss of function approaches of selected signalling pathways (Notch, mTOR and others) and their influence in stem cell maintenance and cell fate decisions during differentiation. Array based determination and modeling of the molecular signals between epithelial stem cells and their microenvironment.

Theme 3: Epigenetics
Studying the role of epigenetic regulators in particular of the polycomb group (Bmi1 andEzh2), for stem cell and cancer stem cell self-renewal.

Theme 4: Dysregulation
Characterization of dysregulated niches and stem cells and analysis of their contribution to diseases with particular emphasis on breast and gastric carcinomas.

Theme 5: Plasticity and Reprogramming
Determination of the genetic program of squamous and related epithelial stem cells, followed by elucidation of the molecular basis of plasticity and mechanism of reprogramming.

i) WP2 Objectives

- Use of stem cell markers combined with genetically engineered reporter mice to isolate and monitor the differentiation potential and gene expression profile of epithelial stem cells and cancer stem cells of different origin.
- Functional tests using gain and loss of function approaches of selected signalling pathways (Notch, mTOR and others) and their influence in stem cell maintenance and cell fate decisions during differentiation. Array based determination and modelling of the molecular signals between epithelial stem cells and their microenvironment.
- Studying the role of epigenetic regulators in particular of the polycomb group Bmi1 and Ezh2, for stem cell and cancer stem cell self-renewal.
- Characterization of dysregulated niches and stem cells and analysis of their contribution to diseases with particular emphasis on breast and gastric carcinomas.
- Determination of the genetic program of squamous and related epithelial stem cells, followed by elucidation and validation of the molecular basis of plasticity and mechanism of reprogramming.

ii) WP2 Results

In this Work Package we aim to identify and compare the cellular and molecular relationships between epithelial stem cells of different origin and in disease states such as cancer. In addition, based on in vivo behaviour of stem cells, we aim to derive and refine mathematical dynamic modeling of stem cells in interaction with their niches. We structured the work package around 5 major themes. Significant progress has been made on all themes, which are summarized below. The main emphasis has been on the generation of the specific conditions and tools in Period 1.

This was followed by in depth analyses in Period 2 and 3. Several of the major highlights are listed in the IMPACT section below.

Theme 1 (cellular hierarchy and stem cell identity):
In vivo lineage tracing using the WNT pathway stem cell marker Lgr5 was extended to gastric epithelium. This demonstrated the importance of this pathway also for gastric stem cells and also showed that these cells are the cell of origin for gastric cancer. Extensive gene expression profiling for intestinal, gastric and hair follicle stem cells further highlighted the WNT transcriptional network in stem cell control. Using mouse models for basal and alveolobular breast cancers based on lesions commonly found in the human equivalents allowed the sorting of tumor-initiating cells (TICs) based on FACS markers. This showed highest numbers of TICs in alveolobular breast cancer and also allowed to study the effects of chemotherapy resistance. For thymic epithelial stem cells Foxn1 was shown to be a critical regulator of their differentiation. Furthermore we showed that squamous epithelia of the Rat contain clonogenic stem cells which are critically regulated by rapamycin/hypoxia/mTOR signaling and can behave as hair follicle stem cells when transplanted in skin.

Theme 2 (signaling and fate choice):
Critical signaling pathways for stem cells mentioned above were functionally analyzed both in vitro and in vivo in new mouse models. This highlighted the importance of both Foxn1 as well as Notch 1 and Notch 2 in regulating skin and mammary stem cells and their role, when deregulated, in cancer. In addition skin-specific ablation of Notch 1 and 2 provided a new model for the disease atopic dermatitis. Based on previous lineage tracing experiments lung epithelial subsets were used to derive a key transcriptional network required for their differentiation. With regard to obtaining a dynamic model of in vivo crypt fission a 3-D agent-based cell interaction model was obtained which included parameters such as elasticity and stiffness. In a second step we specified the gland model in order to obtain a 3D computer model of a growing intestinal organoid in vitro which included Paneth cells as key 'niche' regulators of intestinal stem cells. This leads for the first time to a consistent model of the formation of an intestinal stem cell niche in vitro and allowed simulation of organoid growth in agreement with experimental findings of the Clevers lab.

Theme 3 (epigenetics):
Based on previous observation of importance in hemapoietic and brain stem cells, we investigated using conditional mouse models the importance of key Polycomb transcriptional epigenetic regulators Bmi1 and Ezh2 in mammary and intestinal stem cells and in cancers of these tissues. Surprisingly, no significant role was found for mammary epithelial stem cells or their differentiation for Ezh2 whereas Bmi1 is important. Technical difficulties prevented a firm conclusion as to their impact on diverse breast cancer models. Despite reports of others, deletion of Bmi1 did not impact on intestinal stem cells in vivo, whereas a redundant role for Ezh2 and Ezh1 was found in intestinal stem/progenitor cells

Theme 4 (dysregulation):
Like for the intestine, Lgr5+ stem cells were found in the stomach (pylorus). These cells were also shown to be the cancer initiating cells for stomach adenomas upon APC deletion. We have studied the role of the inflammatory tumour microenvironment on tumour growth, metastasis and therapy response in realistic mouse models of de novo breast cancer. It

was found that the adaptive immune response affected lobular breast cancer but not HER2+ driven breast cancer indicating that
Together, our data indicate that the effects of the inflammatory microenvironment on tumour metastasis and therapy response may be strongly dependent on breast cancer subtype which has important consequences for therapy.

A comprehensive genetic screen for stem cell regulators in Drosophila neuroblasts identified several highly conserved key regulators. Three of these were tested for effects on mammary progenitor differentiation and two (PP4 and TATSF1) were found to have strong differentiation-blocking properties, when inhibited suggesting possible conserved tumor suppressive properties.

Theme 5 (plasticity and reprogramming):
Yamanaka reprogramming factors were tested on adult keratinocytes. Reproducible reprogramming was observed with all 4 Yamanaka factors (OCT4, SOX2, KLF4 and cMYC) or with similar efficiency omitting cMYC. Higher numbers of undifferentiated ES cell-like colonies are obtained than from dermal fibroblasts. However significant variation was observed between iPS clones and only few closely resemble pluripotent ES cells.

The Barrandon and Blackburn groups have demonstrated the isolation and culturing of subpopulations of thymus epithelial stem cells (TECs) from rats. Interestingly, these cells can adopt hair follicle fate when exposed to permissive skin conditions. These cells have been extensively analyzed and their gene expression programs have been identified under these different conditions. This illustrates the importance of specific niche factors in reprogramming stem cells.

iii) WP2 Outputs

Relevant data has been deposited in public databases. Please see individual deliverable reports for full details of these database deposits.

This work package has resulted in the following outputs:
- 19 peer-reviewed research publications
- 5 reviews (one of which is in press)
- 4 patents
- 1 other products
- An additional 10 manuscripts are currently under review or in preparation.

Pluripotent mouse embryonic stem (ES) cells and tissue-restricted adherent neural stem (NS) cells exhibit robust symmetrical self-renewal in simple and well-defined environments and are readily induced to differentiate upon modulation of the culture conditions. In this Work package we aimed to analyse cellular and molecular organisation of these cultivated stem cell lines. The key goals were:

c) Work Package 3: Principles of organisation and potency in cultivated stem cells

Theme 1: Cellular hierarchy

To define whether fluctuating gene expression is "noise" inherent to permissiveness for multilineage gene expression, or reflects hierarchical segregation into functionally distinct sub-populations.

Theme 2: Signalling and fate choice
To delineate the critical signal transduction pathways and downstream transcriptional mediators that cause ES and NS cells to exit self-renewal and enter into lineage commitment.

Theme 3: Epigenetics
To characterise the relationship between signalling pathways and epigenetic regulators, notably Polycomb and Trithorax complexes, during ES and NS cell self-renewal and commitment.

Theme 4: Dysregulation
To elucidate the relationship between recurrent genetic alterations incurred in culture with the normal circuitry of self-renewal and with transformation events in cancer stem cells.

Theme 5: Plasticity and reprogramming
To illuminate how a tissue specific stem cell state can be preserved during extended dormancy or derestricted to pluripotency under the influence of specific transcription regulators.

i) WP3 Objectives
- To define whether fluctuating gene expression is "noise" inherent to permissiveness for multilineage gene expression, or reflects hierarchical segregation into functionally distinct sub-populations.
- To delineate the critical signal transduction pathways and downstream transcriptional mediators that cause ES and NS cells to exit self-renewal and enter into lineage commitment.
- To characterise the relationship between signalling pathways and epigenetic regulators, notably Polycomb and Trithorax complexes, during ES and NS cell self-renewal and commitment.
- To elucidate the relationship between recurrent genetic alterations incurred in culture with the normal circuitry of self-renewal and with transformation events in cancer stem cells
- To illuminate how a tissue specific stem cell state can be preserved during extended dormancy or derestricted to pluripotency under the influence of specific transcription regulators.

ii) WP3 Results

The overarching aim of this Work package is to analyse the cellular and molecular organisation of cultivated stem cell lines and to determine whether microheterogeneity in cultures relates to "noise" inherent to permissiveness for multilineage gene expression, or reflects hierarchical segregation into functionally distinct sub-populations. This important problem is approached by several strategies, ranging from development of novel technologies for cell handling and analysis to genome-wide analysis of gene expression and epigenetic status. The work is divided into five inter-related themes. Significant progress has been made in all themes, and highlights from the work are described below:

Theme 1 (Cellular hierarchy):
Progress towards large-scale handling of cells and colonies has been hampered by the lack of adequate technologies to simultaneously handle and analyze large numbers of individual cell or colonies. Novel

strategies and instrumentation for isolation of single cells and for gene expression in large numbers of single cells has been developed. The CellCelector system provides an unbiased analysis of a colony population, and is combined with a sterility box that provides a well-controlled environment for the ensuing cellular analysis. A system for combined protein and RNA detection has also reached the proof-of-principle stage, allowing for simultaneous analysis of specific model proteins and RNAs, with the aim of turning this technology into the genome- and proteome-wide scale. In Theme 1, the extent and importance of microheterogeneity in stem cells has been addressed in several ways, including studies of Ca2+ oscillations, Notch signaling and Nanog levels. Several new fluorescence-based tools to probe microheterogeneity have been developed, including reporters for Rex1 expression status which specifically marks the ES cell "ground state". Importantly, reduction of microheterogeneity in ES cells in response to pharmacological intervention with Erk and Gsk3 (the 2i condition) has been accomplished. Finally, an improved understanding of X-chromosome dynamics in the early differentiation process has been obtained, and a role for pluripotency factors in controlling Xist and Tsix, which are key regulators of X-chromosome chromatin remodelling, has been demonstrated.

Theme 2 (Signalling and Fate Choice):
Given the fact that the developmental ground state can be robustly achieved through the use of 2i, detailed molecular studies of the ground state and the exit from this state have become possible. In Theme 2, a detailed transcriptome analysis of this transition is reported, and is importantly combined with an epigenetic analysis of chromatin profiles in ground-state and conventional ES cells. The data also indicate that ES cells in 2i are in a relaxed epigenetic context, but that RNA polymerase II, although recruited to promoters, appears to be constrained by promoter proximal pausing. With regard to exogenous factors, novel insights into the role of Notch signaling in early neural differentiation of ES cells has been obtained, and Notch provides different signaling outputs in a temporally controlled cell context-dependent manner. The relation between Notch signaling and the Myc family of genes as well as the Homeodomain transcription factors has been elucidated. A more detailed understanding of the ground state has also been achieved, and a role for GSK inhibition to control Tcf3 levels via beta-catenin has been established. In work extending beyond the original proposal a genome-wide siRNA screen has been implemented to identify mediators of exit from the ground state (PLoS Genetics, in press).

An important aspect of Theme 2 has also been the development of tools to identify genes important for self-renewal and neuronal commitment in NS cells. A series of transposon-based vectors to accomplish both gain- and loss-of-function unbiased screenings of the genome has been developed and the concept validated. Progress in the derivation of specific neuronal cell types of medical interest from NS cells is also reported.

Theme 3 (Epigenetics):
A genome-wide approach to understanding the ES cell ground state has been conducted, in collaboration with the HEROIC FP7 project. It was found that naive pluripotency was characterized by low levels of HeK27me3 silencing, but that this, interestingly, was combined with minimal transcriptional activity of lineage-associated genes. The bivalent state (defined by H3K27me3 and H3K4me3 histone marks) was found to exist not only in conventionally cultured ES cells but also, although to a more limited extent, in ES cells in the ground state. The data also indicate a

role for Polycomb proteins in the lineage specification process rather than in the pluripotent state itself.

Theme 4 (Dysregulation):
Various types of stem cells, including ES, iPS and NS cells, have been suggested for future clinical use in regenerative medicine and cell therapy. Important considerations before using cells in the clinical setting are to eliminate the exposure to undefined human or animal products and to get a thorough understanding of the genomic integrity of the cells. Both these topics have been addressed in Theme 4. Human NS cells have been cultured on a variety of polymers with different surface properties. Laminin turned out to be the preferred substrate, and the NS cells were shown to maintain a self-renewing gene expression signature with little senescence and differentiation. To probe gene expression and genomic integrity, a software called DISCO (Disovery of Sublte Clustered Organisation) was developed, and tested on available high-throughput expression data from mouse and human ES, iPS and NS cell lines. The data suggest that karyotypic changes are not a ubiquitous feature of stem cells in culture.

Theme 5: (Plasticity and Reprogramming):
New insights into the propagation of NS cells, with specific regard to dormancy and potency, have been obtained, and BMP and FGF2 signaling are central to these processes. FGF suppresses terminal differentiation and in the presence of BMP sustains stem cell potency, which allows for the first long-term culture system for rat NS cells. Reprogramming of NS cells to ES cells has been accomplished, and the application of 2i/LIF conditions induced full reprogramming. Similarly, a role for Nanog in reprogramming back to authentic pluripotency has been demonstrated. The interconversion between the EpiSC and ES cell state has also been successfully studied, and introduction of Klf4, combined with ground state culture conditions was sufficient to convert EpiSCs into germline competent iPS cells. A genome-wide screen for novel reprogramming factors has also been completed, and two related nuclear receptor genes, Nr5a1 and Nr5a2, have been identified.

iii) WP3 Outputs

Relevant data has been deposited in public databases. Please see individual deliverable reports for full details of these database deposits.

This work package has resulted in the following outputs:
- 28 peer-reviewed research publications (one of which is in press)
- 8 reviews
- 2 patents
- 6 other products
- An additional 7 manuscripts are currently under review or in preparation.

d) Work Package 4: Regulation of asymmetric cell divisions, self-renewal and the niche

We aimed to define the regulatory pathways and mechanisms governing stem cell self-renewal using genetic, cell biology and molecular approaches in defined tissue specific stem cell systems. The key goals were:

Theme 1: Identifying regulators of cell fate choice in Drosophila, C. elegans and vertebrates
To identify genetic regulators of self-renewal and asymmetric cell divisions in Drosophila and identify and characterise their relevant vertebrate homologues in specific tissues and organs.

Theme 2: Signaling pathways regulating self-renewal
To assess the role of key signaling pathways in cell fate progression in vivo and in established ex vivo paradigms to evaluate how stem cells escape lineage progression and differentiation cues.

Theme 3: Characterising the stem cell niche
To characterise the stem cell niche in haematopoiesis and in skeletal muscle as a regulator of cell fate during development and growth, and after regeneration.

Theme 4: Genetic networks and epigenetic regulation
To assess the in vivo genetic circuitry which directs cell fate decisions and simultaneously maintains the stem cell pool by investigating tissue specific transcriptions factors which are associated with cell fate choice and differentiation (ex. Pax3, Pax7, MyoD) and assessing their links with self-renewal.

i) WP4 Objectives
- To identify genetic regulators of self-renewal and asymmetric cell divisions in Drosophila and identify and characterise their relevant vertebrate homologues in specific tissues and organs.
- To assess the role of key signalling pathways in cell fate progression in vivo and in established ex vivo paradigms to evaluate how stem cells escape lineage progression and differentiation cues.
- To characterise the stem cell niche in haematopoiesis and in skeletal muscle as a regulator of cell fate during development and growth, and after regeneration.
- To assess the in vivo genetic circuitry which directs cell fate decisions and simultaneously maintains the stem cell pool by investigating tissue specific transcriptions factors which are associated with cell fate choice and differentiation and assessing their links with self-renewal.

ii) WP4 Results

Diverse strategies in several model organisms addressed the issue of mechanisms governing self-renewal and commitment of specific stem cell types. In Drosophila neuroblasts, a transgenic RNAi screen identified a near-complete set of regulators of self-renewal versus differentiation. In this stem cell model, quantitative parameters for neuroblast size, number and shape as well as the number and size of differentiating daughter cells were phenotyped thereby linking molecular effectors with cell behaviour. In hematopoietic stem cells in mice, another technological approach was the development of an inducible gene and shRNA expression method to screen for regulators of HSC fate using a novel mouse line with lentiviral transduction to govern robust inducible expression of transgenes or interfering RNAs to HSCs and other hematopoietic cells. This allowed a powerful inducible and reversible gene overexpression or knock-down in resident HSCs in vivo. Other technological breakthroughs include the development of novel biosensors with a variety of functionalities, in particular for a number of specific

signalling pathways. This type of technology permits the analysis of single cell behaviour in a mixed population of cells in real time by videomicroscopy.

In C. elegans, single cell tracking in posterior cells in vivo, and genetic manipulations identified mechanistic roles for a group of distinct transcription factors, notably those playing critical roles also in vertebrates, in the process of asymmetric cell division. In addition Wnt signalling was linked with cell fate identities. The mechanism of action of other potent regulators of cell fates, some of which are implicated in human diseases such as CADASIL, were investigated, and in some cases bona fide targets that have been elusive were identified. Significantly, links between oxygen tension and Notch were established for arterialisations, and for temporal cell fate specification during development in the muscle lineage. Oxygen tension or Notch activity were also linked to the regulation of distinct cell states that haematopoietic and skeletal muscle stem cells can adopt during homeostasis as well as after extreme environmental stress in mouse and humans.

Investigation of the niche as well as the regulators of stem cell activity are of major importance. Here, experimental perturbations of the niche was done in blood and muscle. Lymphopoiesis in the former and stem cell quiescence in the latter were altered by modulation of transcription factors or cell to cell signalling. Transcriptome analysis is underway to identify novel regulators of the dormant/quiescent cell states with extrinsic influences provided by the niche. In some cases, for example skeletal muscle, upstream transcription factor targets were identified, either as downstream protein effectors, or small non coding RNAs which play important roles in regulating the self-renewal to commitment transitions. Finally, transplantation of blood and muscle stem cells provided additional experimental models for functional readouts. Of interest, transplanted stem cells were found in some cases to behave radically differently compared to the equivalent endogenous stem cell entity.

iii) WP4 Outputs

Relevant data has been deposited in public databases. Please see individual deliverable reports for full details of these database deposits.

This work package has resulted in the following outputs:
- 25 peer-reviewed research publications
- 7 reviews
- 0 patents
- 3 other products
- An additional 4 manuscripts are currently under review or in preparation.

e) Work Package 5: Development and implementation of an integrated data management and analysis platform

Within EUROSYSTEM a huge number of qualitatively different deliverables such as experimental and simulation data, experimental protocols, and statistical / bioinformatical analysis tools as well as project specific (administrative) documents have been generated. To facilitate an effective, consortium-spanning use of all these different components, an integrated data management and analysis platform has been developed and

implemented. Such a platform also provides a number of theoretical methods that enable sophisticated data analyses as well as an efficient knowledge management. The integrated platform particularly links the different experimental results and the theoretical tools for a common, consortium-spanning application. The specific objectives of this WP have been the development and implementation of:

- a multifunctional data management system with internet portal providing interfaces for the access of local data bases and the (remote) use of analysis methods
- a formal stem cell ontology including a common annotation scheme and an integrated data storage concept for experimental and simulation data
- new specific methods for the statistical analysis of high dimensional molecular data using
  multivariate approaches based on quantitative expression values including hybrid strategies (i.e. complementary use of different data types)
- single cell tracking procedures that allow the automatic reconstruction and the statistical analysis of cell fate trees (i.e. cellular genealogies) based on time lapse video data

Theme 1: Data Integration
Development and implementation of a comprehensive database management system and internet portal.

Theme 2: Multivariate analysis methods for molecular expression data
Development of new specific methods for the statistical analysis of high dimensional molecular data using multivariate approaches based on quantitative expression values.

Theme 3: Single cell tracking
Development and implementation of a single cell tracking and analysis tools.

Theme 4: Stem cell ontology
Development of a stem cell ontology including a common annotation scheme and data storage concepts for experimental and simulation data.

i) WP5 Objectives
- Development and implementation of a comprehensive database management system and internet portal.
- Development of new specific methods for the statistical analysis of high dimensional molecular data using multivariate approaches based on quantitative expression values.
- Development and implementation of a single cell tracking and analysis tools.
- Development of a stem cell ontology including a common annotation scheme and data storage concepts for experimental and simulation data.

ii) WP5 Results

The research in this work package centered around the following four themes: 1. data integration, 2. multivariate analysis methods for molecular expression data, 3. development and implementation of a single cell tracking and analysis tools, and 4. stem cell ontology. All the deliverables generated under themes 1 to 4 contributed to the establishment of an extensive set of tools for integrated data management and analysis. In particular, we provide (as our final WP5 deliverable) a

substantially improved StemDB resource. Beside a considerably extended functionality for project data management, StemDB has been extended to handle a couple of new data types. For some of them (e.g. single cell genealogical data) currently no other web-based data-base is available. Beyond this, we developed a number of specific data analysis methods, which are all somehow linked to StemDB (e.g. via the newly implemented BioResource Index).

In the following, we will describe the obtained results theme by theme:

Theme 1: We started by formulating a data integration concept, which served as a guideline for the development and extension of the EUROSYSTEM data and project management tool StemDB. Based upon this concept we adapted and extended the StemDB tool to account for new administrative and scientific requirements and released a new version of StemDB, available at http://www.stemdb.org (D5.2). According to the advice of the Scientific Advisory Board, the focus of the further StemDB development had been redefined towards an improvement of the data and project management functionality as well as towards the inclusion of easily assessable, pre-evaluated information on analysis methods and tools instead of providing a comprehensive data-base for experiential results. Following up on this, we integrated an easy to use quick search functionality and the BioResource Index, which provides information on many commonly used bioinformatics and systems biology databases and software tools (D5.8). Additionally, we extended the StemDB functionality to handle analysis of next generation ultra-high throughput sequencing (UHTS), which has been implemented in the GeneProf module (D5.14).

Theme 2: The work in this theme centered on the development of multivariate data analysis methods for high-dimensional molecular data. To facilitate a more reliable analysis of transcript expression in terms of providing estimates of absolute expression levels, we developed a new bioinformatical method, called "hook-method" which has been implemented into a software tool available in a C++ as well as a Java implementation. Both versions are publicly available at http://www.izbi.uni-leipzig.de/downloads_links/programs/hook.php and linked to the BioResource Index of StemDB (D5.4). Beside a correct quantification of individual measurements, analysis of high-dimensional gene expression data requires methods that allow for understanding relations and interactions of the individual measurements. Here, the analysis of interelated gene sets, causal networks as well as genetically correlated gene activities (eQTL) can be used to discover important gene relationships as well as cause-effect associations. Specifically, we developed a method to identify gene sets, which are testes for their statistical significance by means of the Westfall-Young principle based on re-sampling or by the parametric method of spherical tests. The method has been implemented in a R-package and is publicly available at http://www.people.imise.uni-leipzig.de/maciej.rosolowski/software.html (D5.5). Another class of methods to identify functionally related gene sets among genes are gene enrichment procedures. These methods are able to identify functional annotation terms that are overrepresented in a list of differentially expressed genes. We demonstrated how it is possible to use integrated gene expression analysis methods (in particular a novel meta-analysis method that is based on a biclustering principle together with a Bayesian integration method) to dynamically generated gene sets that can be used for enrichment analysis to augment detailed manually curated annotation (D5.5). The GeneNet method developed under this theme allows constructing causal gene networks from gene

expression data. The algorithm has also been implemented as an R-package and is publicly available at http://www.strimmerlab.org/software/genenet/index.html. Another method to understand molecular interactions is the eQTL methodology, which combines genetic and genomic information. This method (which is publicly available at http://webqtl.org, also assessable from within StemDB BioRessource Index) has been applied to evaluate genome-wide RNA transcript expression levels in purified Lin-Sca-1+c-Kit+ multi-lineage cells, committed Lin-Sca-1-c-Kit+ progenitor cells, erythroid TER-119+ cells, and myeloid Gr-1+ cells, isolated from the bone marrow of approximately 25 genetically related and fully genotyped BXD – C57BL/6 (B6) X DBA/2 (D2) – recombinant inbred mouse strains. These methods have been used to identify and analysis network structure underlying the regulation of stem cell differentiation (D5.9).

Theme 3: Another branch of new bioinformatical methods for application in stem cell biology is the reconstruction of individual cell fates from time lapse video data of living cells. Here we developed a data structure that enables an efficient storage of genealogic information, which can be extracted from time lapse video data (D5.1). Moreover, we developed and implemented an algorithm that is able to automatically track individual cells in vitro and to reconstruct cellular genealogies (i.e. annotated tree structure describing the development of individual cell in a clonal level) (D5.3). The newly developed automatic tracking algorithms have been successfully applied to in vitro cultures of haematopoietic stem and progenitor cells. Because for cellular genealogies no standard statistical analysis methods are available, we developed and investigated a number of topological measures, which can be used to statistically quantify and compare different cellular genealogies. These measures have been incorporated into a software called gAnalyzer, which is a C++ tool for the numerical evaluation, the statistical analysis, and the graphical representation of single cell tracking data and the resulting cellular genealogies (D5.6). The above described methods for automatic single cell tracking are provided as a tool kit (programmed in "Mathematica"). Together with sophisticated methods for an efficient post-processing, they have also been incorporated into the software tool ("CellTracker"), which has been generated in this project (D5.11). We also tested the developed methods for automatic single cell tracking for their application to mouse embryonic stem (mES) cell cultures. However, it turned out that the quality of the video data generated by the "Biostation" microscope (which was available in Cambridge) was not sufficient to successfully read out information of the single cell level. This result had two consequences: First, we decided to include also the analysis of mES cell colonies (not only single cells) and, second, we partially transferred the imaging experiments to the University Dresden, where we could use the "DeltaVision" microscope, which provided data with a higher quality (D5.10).

Theme 4: As a last point we paved the way for the development of a domain specific (formal) stem cell ontology by a detailed ontological analysis of the stem cell biology field. The core ontology for the stem cell domain has been further developed to include particularly two levels. The first level exhibits a Simple Process Object Ontology (SPOO), which is the basis for coherently handling of objects, processes, and the properties associated to them. This ontology is utilized for the development of the second level, which captures the essential features of stem cells in terms of cellular genealogies (see Theme 3). This ontological analysis has been used to develop an annotation scheme for

the description and data base representation of cellular genealogies within StemDB, which has be further refined, on the basis of the developed formal cellular genealogy ontology "CGO" (D5.7). Based on the developed formal stem cell ontology and the annotation scheme that is based upon this formalism, we extended the functionality of StemDB by the ability to handle time-lapse video date for single cell tracking and for cell genealogical data that is generated from this (D5.13).

iii) WP5 Outputs

Relevant data has been deposited in public databases. Please see individual deliverable reports for full details of these database deposits.

This work package has resulted in the following outputs:
- 11 peer-reviewed research publications
- 0 reviews
- 0 patents
- 11 other products
- An additional 4 manuscripts are currently under review or in preparation.

f) Work Package 6: Training, Federation and Outreach

This Work Package addressed the requirements for achieving an open, attractive and competitive European research environment in fundamental stem cell biology. Building on knowledge gained in the Sixth Framework Programme (FP6) integrated project EuroStemCell, we proposed an ambitious programme that promoted efficient exchange of intellectual and methodological expertise within the consortium trained and supported a cadre of first class stem cell scientists and developed active, innovative and effective public engagement throughout Europe. In addition, we proposed to begin the process of formal federation of the European stem cell research field, through the establishment of an active pan-European network of established and emerging basic stem cell researchers working in different tissues and organisms.

WP6 therefore had three themes, whose specific aims were as follows:

Theme 1: Training Programme
- Facilitate collaborative exchange and technology transfer between participating laboratories.
- Foster engagement between stem cell researchers and computational and mathematical biologists
- Provide high-level training in theoretical stem cell biology, computational and mathematical biology, and specialist technologies required to advance the field.

Theme 2: Federation of the European stem cell research field
- Constitute a broadly-based pan-European network of excellence in fundamental stem cell biology, composed of leading established and emerging investigators.

Theme 3: Implementation of an outreach programme on stem cell research across Europe
- Implement a pan-European outreach programme on stem cell research and related societal and ethical issues, for including the wider scientific and lay communities in dialogue about stem cell biology.

The overall anticipated outcome of this work was increased cohesion and competitiveness in European stem cell research.

i) WP6 Objectives

- Facilitate collaborative exchange and technology transfer between participating laboratories.
- Foster engagement between stem cell researchers and computational and mathematical biologists.
- Provide high-level training in theoretical stem cell biology, computational and mathematical biology, and specialist technologies required to advance the field.
- Constitute a broadly-based pan-European network of excellence in fundamental stem cell biology, composed of leading established and emerging investigators.
- Implement a pan-European outreach programme on stem cell research and related societal and ethical issues, for including the wider scientific and lay communities in dialogue about stem cell biology.

ii) WP6 Results

This Work package set out to deliver the broad training, federating and outreach aims of EUROSYSTEM, addressing the directive in the original call for projects for the project to be "a federating force in the field … by developing common standards for research, by promoting training and education and encouraging outreach activities." As documented above, we have delivered a comprehensive and ambitious programme addressing all of these requirements and, through the activities performed, have met and surpassed this directive. At the outset of the project, we envisaged that the overall impact of the work package would be to increase cohesion and competitiveness in European stem cell research. We believe that the work performed has fulfilled this aim:

In Theme 1 of the work package, we established an extensive training programme aimed both at consortium members and more broadly, at European stem cell scientists. This was structured both to develop common standards in research and to promote training, by providing a suite of different training activities aimed at each of these requirements. Thus, we established an effective scheme to support training exchanges between the laboratories participating in EUROSYSTEM, with the aim of promoting technology transfer between laboratories and of facilitating joint projects – and the further aim of developing common standards in research practice. This scheme was extended to include the network of associate consortium members also established as part of this work package. Through this scheme, we supported 29 exchange visits. Most of these exchange visits were made by early career stage researchers, and therefore benefited those supported by extending their skills repertoire, exposing them to a new intellectual environment and providing important networking opportunities. Many of the exchanges made were between laboratories whose primary expertise was in computational or systems biology and those working in basic stem cell biology, and therefore supported transfer of knowledge of the methodology and potential of systems biology to stem cell research. In addition, we developed and ran a series of workshops aimed at training stem cell researchers in new ways of working, including computational and systems biology approaches, analysis of large datasets generated using high throughput sequencing and proteomics technologies, and in techniques for single cell-level analysis needed for advancing mechanistic understanding of stem cell biology.

Again, these were mainly targeted at early career stage researchers, and thus served both to bring understanding of these techniques into the consortium laboratories and to equip the participants with the skills need for a competitive research career in academia or industry as biology increasingly adopts these approaches. These workshops were attended by a total of 131 participants. We also established a workgroup on the Biology of Neural Systems; this was constituted to provide a forum for in depth discussion of this field, with the aim of catalyzing progress. Finally, we hosted – together with two other FP7-funded stem cell consortia – four summer schools on stem cell biology and regenerative medicine, which provided high-level theoretical training to early career researchers drawn from within and outside the consortium; over 216 participants benefited from this initiative in the duration of the EUROSYSTEM consortium, with overwhelmingly positive feedback. We also provided organizational support for a fifth summer school, bringing the total number of beneficiaries from this activity alone to over 270 early stage researchers. We also played a leading role in organizing three major European conferences on stem cell research – the Advances in Stem Cell Research series.

In Theme 2 of the work package, we directly addressed the requirement to provide a federating force in the field. We developed a bespoke, searchable contact database, STEMdirect, for managing stem cell researcher information, and populated this database via a mapping exercise aimed at identifying active stem cell researchers' throughout Europe. In parallel with this effort, we made three open calls for membership of a network of associate Principal Investigators (PIs), aiming specifically to identify excellent researchers active in the stem cell field but at an early stage in their independent career, and stem cell researchers active in areas complementary to those included in the original consortium. Overall, 40 associate PIs were selected, with care being paid to distribution of membership across Europe; most of these PIs were not previously known to consortium members. The resulting group, the European Stem Cell Group, had access to all of the platform technologies generated by the consortium and participated fully in our training and exchange programme. In addition, we held a closed workshop for full and associate PIs each year after constitution of the associate PI network, which provided an important forum for networking and for critical discussion of data, and resulted in initiation of many new collaborations. This initiative was viewed as important and highly successful by all concerned. The grouping is well positioned to take forward a European Stem Cell Network, and also to develop new networks for application for EC funding under the final call of FP7 and in Horizon 2020.

Collectively, the work performed in these Themes has resulted in increased cohesion and competitiveness in European Stem Cell Research.

Theme 3 of the work package addressed the requirement to encourage outreach activities. We identified that the time taken to prepare ideas/materials for outreach activities was a limiting factor in scientist participation. Therefore, this theme focused on developing activities to support outreach events - aimed at a variety of different audiences and types of events – and on training scientists in approaches to public engagement with different audiences, and in use of the tools developed in this project. Specifically, we developed, piloted and refined a series of resources designed to illustrate key concepts in stem cell biology to secondary school students, including a lesson plan

developed in conjunction with teachers; a short educational film on induced pluripotent stem cells; and a game – designed for use with adult audiences at science festivals – aimed at promoting discussion about stem cells and their potential uses. We then prepared a "Stem Cell Outreach Toolkit" using these resources, in conjunction with resources generated in the EU-FP6 integrated project EuroStemCell and the feature length documentary "Stem Cell Revolutions: vision of the future" which was co-funded by EuroStemCell and the Wellcome Trust. We have supported many of the participating laboratories in outreach events using these resources, and additionally have provided hands-on training in use of the "Toolkit" components in a variety of outreach settings to EUROSYSTEM members in each of the partner institutions. As a legacy of the project, and to encourage continued participation in outreach and public engagement, each EUROSYSTEM lab has been provided with a physical copy of the "Toolkit".

In addition, we supported continued development of the website http://www.eurostemcell.org. This website has now developed into a major European Stem Cell information portal. The number of visits has increased from approximately 18000/year at in 2008 (the start of the EUROSYSTEM project) to over 200,000/year in 2012 - from more than 190 countries worldwide. It is growing steadily across all metrics – indicating that an audience and interested community is building around the site, and around stem cell research in Europe. Many EUROSYSTEM consortium members have contributed to the site, with content ranging from fact sheets to commentaries, images, outreach resources and blogs.

The work undertaken in this theme has thus achieved the goal of encouraging outreach activities, and additionally has fulfilled an important role in building capacity in outreach and public engagement in stem cell research, and establishing a network of stem cell scientists active in public engagement.

iii) WP6 Outputs

This work package has resulted in the following outputs:
- 2 peer-reviewed research publications
- 3 other products and the EUROSYSTEM website http://www.eurosystemproject.eu
- A bespoke fully searchable database, STEMdirect, for collation and management of information on stem cell researchers throughout Europe.
- A short educational film on iPS cells which is available for download and will also be added to the "Stem Cell Stories" DVD set.
- 'All about stem cells', a set of downloadable tools and instructions for running a workshop with secondary school students. We have also produced a physical version of our "toolkit" for outreach and public engagement in stem cell research for each of the Partner laboratories, and have trained at least one representative in each partner institution in use of this toolkit.
- A game for engaging the public with stem cell research

Potential Impact:
By bringing together the leading European research teams in an integrated programme, EUROSYSTEM has raised international awareness of European stem cell research. The results of the project include: new and fundamental knowledge; computational integration of pre-existing and novel knowledge; advanced technology platforms, database resources and software tools. These results are reported in 128 scientific publications (including two in press) and have had a significant impact on stem cell research globally. In addition EUROSYSTEM has actively promoted interaction and networking between stem cell investigators and provided various training and meeting opportunities. An overall impact is that the European stem cell community is now well connected and prepared for the next research challenges, including investigations at the high-dimensional systems level.

As a fundamental research collaboration, EUROSYSTEM has no direct socio-economic or societal implications. However, as a consortium of socially responsible scientists cognisant of public interest in stem cell biology, EUROSYSTEM has invested time and resources in developing a major WEB Portal at http://www.eurostemcell.org . This WEB project, which was initially led by EUROSYSTEM, has attracted support from other EC stem cell consortia and further support from an EC concerted action grant. The site has a diverse range of information for different groups and attracts greater than 200,000 visitors per year. EUROSYSTEM investigators have contributed significantly to Information Sheets and FAQs on different aspects of stem cell biology.

a) Impacts and potential impacts from the individual workpackages are summarised below.

i) Work package 1: Towards a systems approach for haematopoietic stem cell differentiation

- Definition of the human lympho-myeloid grimed progenitor. This study 're-routes' the human haematopoietic system and consequently constrains the candidate regulatory transcription factor topologies that instruct cell fate. This cell type is also a key transformation target in human leukaemia.

- A series of integrated data sets describing haematopoietic stem, progenitor and mature effector cells in both mice and humans. These data sets are a rich source of quantitative data for future systems biology. Their linkage to specific cell stages allows integration of 'omics' data with cell type transitions in cell based modelling approaches.

- Discovery of novel regulators of the blood system and determination of their function. Gene discovery and in depth knowledge of gene function remains a central goal of modern biology and development of new or existing pathways paves the way for rational therapy of blood diseases including cancer.

Overall the stage is set for full scale development of systems level studies in haematopoiesis.

ii) Work package 2: In vivo analysis of molecular programs underlying normal and diseased epithelial stem cells

- Using lineage tracing to unambiguously identify Gpr49/Lgr5 cells as active gastric stem cells. This highlights the importance of WNT signaling for self-renewal of diverse types of epithelial stem cells as well as cancer stem cells, which has major consequences for future regenerative medicine studies and cancer biology and therapy.

- Using advanced mouse models to study cancer and cancer stem cells in vivo and assay their importance for resistance to cancer therapies. Cancer stem cells—or CSCs—are presumed to have similar capabilities as healthy stem cells: they can regenerate and differentiate into any cell that makes up the cancer. Such cells are often blamed for relapses in patients who by all other measures appear to have been cured.
- Demonstrating the importance of physical parameters on stem cell behavior which gives a fundamental basis to a careful monitoring of stem cell cultures. Partner 4 (EPFL) has started in March 2011, a spin-off company (gyMETRICS) that will provide the scientific and the stem cell community with tools to monitor several key parameters including pH, temperature and glucose in real time. This could have a particularly important outcome for stem cell production, quality control and regulatory affairs required for regenerative therapies.

- Demonstrating the importance of NOTCH signaling and metabolic pathways as 'niche' signals controlling stem cells in diverse tissues. In the skin Notch signaling induces terminal differentiation of skin stem and progenitor cells and negatively regulates inflammation. It functions as a tumor suppressor. In contrast, in the mammary epithelium, Notch exerts oncogenic properties by increasing glycolysis and inducing pro-tumorigenic inflammation. This opens new ways to find drugs that affect these regulatory pathways which can be of great benefit to treat diseases such as atopic dermatitis and diverse forms of cancer.

- Dynamic modeling of intestinal stem cell behavior and crypt fission in vivo We provide for the first time a comprehensive, agent-based 3D model of intestinal tissue that is capable of describing both stem cell differentiation and tissue morphogenesis. Regulatory mechanisms like that suggested in the project linking differentiation pathways and tissue morphology can be expected to represent general mechanisms in gland formation. Several in vitro gland formation models have been established including such for gastric glands, salivary glands, thyroid glands and mammary glands. The individual cell-based model presented here has a broad range of applications for which systematic experimental support can be provided.

iii) Work package 3: Principles of organisation and potency in cultivated stem cells

- Using genome-wide transcriptional and chromatin profiling to understand gene regulation and the epigenetic landscape in ES cells. This highlights important principles for how pluripotent cells can remain in an undifferentiated state, and how exit to various differentiation lineages can be controlled.

- Using intrinsic and extrinsic factors to unravel principles for transitions between various stem cell states. This highlights the unraveling of novel mechanisms for how the transition between cell states such as ES, EpiSC and NS cells is controlled, and delineating the role of factors such as Nanog, klfs and Nr5 in this process.

- Developing novel tools and technologies to advance handling and analysis of stem cells. Here, a set of new technologies for handling and characterization of single cells and cell colonies has been developed. This has been accompanied by development of technology for simultaneous analysis of RNA and protein in cells, and for quantitative proteomics in ES cells.

iv) Work package 4: Regulation of asymmetric cell divisions, self-renewal and the niche

In summary, all critical objectives have been achieved in this work package:

- Identification of novel regulators of asymmetric and symmetric cell divisions, critical to the fate outcome of the stem cell - important for determining why stem cells are not exhausted.

- Establishment of links between cell-cell signalling, environmental conditions (oxygen tension, necrosis, severe stress), metabolic activity and transcription factors that regulate cell fates - critical information for use of stem cells in clinic.

- Development of new tools and biosensors to modulate stem cell behaviour in vivo, or track their fates in vivo, notably for specific signalling pathways with single cell resolution. This permits resolving molecular outputs from background noise.

- Identification of links between transcription factors and small regulatory non coding RNAs, both of which work in concert to regulate stem cell behaviour - of interest if small ncRNAs will be used as therapeutic tools.

The studies outlined have resulted in numerous publications, most of them in high impact journals. In each of the model systems, from C. elegans, Drosophila to mouse and human, fundamental issues dealing with the observation of single cell behaviours, novel stem cells states, symmetric and asymmetric cell divisions and the role of extrinsic influences and intrinsic cell fate determinants, critical transcription factors and their functional roles were elucidated. These studies represent major advances in understanding the molecular and cellular events that are critical for normal growth and regeneration after injury. As such, they provide a solid framework for designing rational pre-clinical strategies by providing tools for the isolation and identification of relevant cell types for transplantations, and well as the molecular regulators of these cells in the endogenous and transplanted scenario.

Defining the functional properties of stem cell, and their capacity to construct organs and tissues during normal growth and after trauma, are major objectives in regenerative biology and medicine. Although much is known about differentiated tissues themselves, how ancestral stem cells yield diverse effector cell types remains largely unknown. In this project, diverse strategies in several model organisms addressed the issue of mechanisms governing self-renewal and commitment of specific stem cell types. In each of the model systems, from the fly and worm, to mouse and human, the molecular and cellular properties of diverse single cell types were elucidated in normal and pathological conditions.

Major discoveries include the identification of novel states that stem cells can assume under different physiological conditions. In addition, how these cells yield similar or distinct daughters was examined in detail and links were established along the communication network from outside the cell in its niche, to powerful regulatory molecules that alter the fate of the cell. These studies represent major advances in understanding the molecular and cellular events that are critical for normal growth, and regeneration after injury. As such, they provide a solid framework for designing rational pre-clinical strategies by providing tools for the isolation and identification of relevant cell types for transplantations, and well as the molecular regulators of these cells in the endogenous and transplanted scenario.

v) Work package 5: Development and implementation of an integrated data management and analysis platform

- Extended version of StemDB as a sophisticated data management system. The integrated handling and management of cross-disciplinary data is a highly complex and non-trivial task. With StemDB we further developed a web-based system that combines project management facilities with the management and storage of scientific results and data. As the system can be used by the project administration as well as by the scientists of cross-disciplinary projects (storage of results, data, and annotated project-related information) it ensures a highly efficient way to administer and handle scientific results.

- Toolkit for the analysis of high dimensional molecular data. The analysis of high dimensional molecular data (using e.g. gene expression arrays or next generation sequencing methods) has been established as a major tool in cell biology during the last couple of years. The highly dynamic technological development in this area led to an extreme amount of experimental data that needs to be analyzed. Here, EUROSYSTEM WP5 contributed with a number of sophisticated methods that address specifically the identification of sets of co-regulated genes and of gene regulator networks from high-throughput data. These methods are not restricted to the data generated in EUROSYSTEM and have already successfully been used in numerous other contexts.

- Automatic single cell tracking methods. It becomes more and more visible that the identification of many mechanisms that are responsible for the regulation and control of stem cell fate decisions need information on the single cell level. Even more, to understand the system dynamics it is necessary to observe individual cells continuously over time. Due to modern imaging techniques it is possible to obtain time-lapse video data of cell culture systems that in principle provide such data. However, the simultaneous extraction of quantitative data of multiple single cells from the videos is an extremely labor-intense task. Therefore, reliable automatic single cell tracking algorithms and corresponding software implementation are required. Currently, no standard methods or software that is able to fulfill this task is available. Within EUROSYSTEM WP5 we were able to develop a toolkit for automatic segmentation and tracking of single cells cultured in specific in vitro systems. In particular, we applied these methods to track haematopoietic stem and progenitor cells (HSPC) in a biomimetic environment. Also, we could show that our algorithms can be applied to other cell types, such as mouse embryonic stem cells, where we used the methods to automatically track and quantify the process of cell colony growth, morphological change, and fusion over time.

- Description of stem cell genealogies in the context of formal ontologies. To the best of our knowledge the developed ontology framework is the first and the only work devoted to the representation of data on cellular genealogies. The framework is currently being extended to support mappings to other existing biological ontologies, in particular to Cell Type Ontology. Our Cellular Genealogy Ontology (CGO) was developed to represent the needs of automated single-cell tracking. Specifically, we aimed towards establishing the ontology as the basis for a primary annotation scheme for single cell tracking / cellular genealogy data. Using this framework we could already demonstrate that it can be used for a wide spectrum of applications related to representing and structuring data on time-lapse experiments and cellular genealogies. In particular, it can be utilized in object oriented software engineering (model construction) or database schema construction. In addition the developed ontology serves as an annotation schema and a data exchange format and represents the basis of the StemDB CellTracker module which has been developed to upload, annotate, store, and retrieve time lapse video and cellular genealogy data.

vi) Work package 6: Training, Federation and Outreach

- A Training Exchange Scheme. This scheme allowed efficient transfer of technological know-how and experimental methodologies between EUROSYSTEM laboratories, and further supported development of collaborative projects. It thus contributed substantially to the scientific success of the project, and to the wider goal of maintaining competitive edge in the European Stem Cell research sector.

- A series of hands-on workshops in new ways of working – including computational and systems biology approaches to stem cell research, analysis of large datasets generated using high throughput sequencing and proteomics technologies, and techniques for single cell-level analysis relevant to stem cell biology.

These workshops provided advanced training to 131 EUROSYSTEM researchers, largely in early career stages. Importantly, they constituted the first systematic programme designed to broker the interface between stem cell research and systems biology.

- A series of five European summer schools in stem cell research and regenerative medicine, and three European conferences, "Advances in Stem Cell Research". These events, open to EUROSYSTEM members and researchers external to the consortium, provided outstanding training and networking opportunities to European stem cell scientists. Provision of events of this quality in Europe is essential to maintain and increase the visibility of European research – including to early career stage researchers looking for their next career move – and thus to increase competitiveness of the European Stem Cell research field.

- Constitution of the European Stem Cell Group, a group of 40 researchers active in fundamental stem cell research, and initial consolidation of this group through two annual meetings and an Advances in Stem Cell Research conference.

In constituting this group, we successfully identified existing and emerging PIs active in fundamental stem cell research throughout Europe, and promoted extensive interactions within this grouping and between

members of the group and EUROSYSTEM consortium members. This was viewed as an important and highly successful initiative by all concerned. The grouping is well positioned to take forward a European Stem Cell Network, and also to develop new networks for application for EC funding under the final call of FP7 and in Horizon 2020.

- Engagement of European Stem Cell Researchers with European publics through provision of high quality resources ("Stem Cell Outreach Toolkit") for outreach and public engagement, and a consolidated training and support programme for engaging EUROSYSTEM researchers in outreach.

These initiatives have fulfilled an important role in building capacity in outreach and public engagement in stem cell research, and establishing a network of stem cell scientists active in public engagement.

- Supported development of http://www.eurostemcell.org as a major European Stem Cell Information portal. The website eurostemcell.org has developed as a major information resource for European publics wishing to access accurate, accessible and up-to-date information on stem cell biology and regenerative medicine. It also serves to focus scientists' outreach efforts, avoiding duplication and ensuring longevity of contributions. Its high standing relies on its independent status, and on the scientific credentials of its contributors. Therefore, it is vitally important that consortia such as EUROSYSTEM contribute to its development.

b) Main Dissemination Activities

As a fundamental research project the main outlet for EUROSYSTEM results is publication in peer-reviewed international journals. In addition, however, EUROSYSTEM adopted a pro-active approach to target different user groups through a multi-level dissemination strategy. In particular the project fostered and made wide use of EuroStemCell (see http://www.eurostemcell.org online) for WEB dissemination and employed a part-time communications officer to achieve maximum visibility and outreach impact.

Dissemination routes included:
- Peer-reviewed scientific publications in leading international journals
- Presentations at European symposia and international conferences
- Open access database resources
- Availability of results, methods and protocols, for Masters and PhD programmes of all the university partners
- Training courses and workshops
- Summer Schools including faculty and students from outside the consortium
- Outreach materials to inform and engage the lay public
- Educational and role play materials for school children and college students
- Information and briefings for policy-makers, regulators and administrators
- Dialogue with patients and other interest groups
- Public web site with answers to frequently asked questions and provision of both general and specific information
- Media communication – press releases, media briefings, glossaries, facts sheets, spokespersons
- Production and release of new products for stem cell research by small and medium-sized entreprises (SME) partners

- Dialogue with bioindustry associations and biopharmaceutical companies
List of Websites:
http://www.eurosystemproject.eu/
Project Co-ordinator Contact Details: Professor Austin Smith, Wellcome Trust - Medical Research Council Stem Cell Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR,
Tel: +44 (0)1223 760233, Fax: +44 (0)1223 760241, E-mail: ags39@cscr.cam.ac.uk
Project Office Contact Details: EUROSYSTEM Project Office, Wellcome Trust - Medical Research Council Stem Cell Institute, University of Cambridge, Tennis Court Road,
Cambridge CB2 1QR

Tel: +44 (0)1223 760288,
Fax: +44 (0)1223 760241,
E-mail: eurosystem-office@cscr.cam.ac.uk