

# Stem Cells and Cancer Group



Christopher Heeschen

## Group Leader

Christopher Heeschen was born in 1966 in Kiel (Germany). Following his university studies in Budapest, Munich and Berlin, he obtained his MD in 1997 at the *Universität Hamburg* (Germany). After three years' clinical training in Internal Medicine at the same university, he joined the Falk Cardiovascular Research Center at Stanford University (USA) in 1999 where he worked on basic mechanisms of angiogenesis and vasculogenic stem cells.

He obtained his PhD in 2001 and subsequently joined *Johann-Wolfgang-Goethe Universität* in Frankfurt/Main as Staff Scientist (he was promoted to Junior Group Leader in 2003). He became an independent investigator in 2004 as Professor of Experimental Oncology and Transplantation and Head of the Department *Experimentelle Forschung Chirurgie* at *Ludwig-Maximilians-Universität* in Munich (Germany).

In 2008 he was appointed as Professor of Medicine at the *Université Montpellier I* (France) and Department Head for *cellules souches somatiques au cours de développement de l'homéostasie et de la régénération tissulaire*. Since 2009, he has been working at the CNIO as Senior Group Leader in the newly founded Clinical Research Programme.

Christopher Heeschen has published more than 90 articles in prestigious scientific journals such as the *New England Journal of Medicine*, *Nature Medicine*, *The Lancet*, *Cancer Cell*, *Cell Stem Cell*, *the Journal of Experimental Medicine*, and *PNAS*, among others. He has made major original contributions to the field of stem and vascular cell biology that have advanced our understanding of the basic processes of stem cell function and trafficking. His research has been recognised through numerous international awards (most recently the Richtzenhain Award from the German Cancer Research Centre) and has earned him an ERC Advanced Investigator Grant for pancreatic cancer research.

## Summary

We have demonstrated that growth and progression of pancreatic cancer, the deadliest solid cancer, is mediated by tumour-initiating cells that bear the physiological properties of stem cells.

We are now conducting extensive *in vitro* and *in vivo* studies to thoroughly characterise the defining features of these tumour-initiating cells. We combine state-of-the-art *in vivo* studies using mouse models with comparative genomic and proteomic analyses of single cells to gain new insight into their regulatory machinery. These data are validated by direct comparison with freshly isolated human cells from tumour specimens as well as the circulating blood using microfluidics technology.

We ultimately aim to develop novel targeted therapies to specifically eliminate these cells as the root of pancreatic cancer. Targeted delivery of new therapies in combination with advanced imaging technologies will be achieved by nanoparticle technology.

## Strategic Goals

- Elucidate the functional properties of pancreatic cancer stem cells (CSCs) *in vitro* and *in vivo*
- Identify *in vivo* the cell of origin for pancreatic cancer stem cells
- Develop novel pancreatic cancer stem cell-based therapeutics
- Validate novel treatment modalities in preclinical models of pancreatic cancer
- Clinically translate novel treatment modalities to patients with pancreatic cancer



**Staff Scientist:** M. Michela Mancarelli (since November). **Post-doctoral fellows:** Jenifer Clausell (since September), Jorge Dorado, Alicia González (until June), Patrick C. Hermann, Enza Lonardo and Michele Petruzzelli (since September). **Graduate students:** Sonu Bhaskar (February-April), Michele Cioffi, Olivia Garandeau (until June), Kristina Jucikaite (until June) and Irene Miranda. **Technicians:** Sonia Alcalá, Mercedes Alonso, Javier Álvarez, Mario Bautista, Ildiko Meny (since October) and Iñaki Merino (July-August).

## Highlights

### **In vivo characterisation of pancreatic cancer stem cells**

Previous studies from our laboratory have now set the stage for our future projects as we aim to definitively establish the cancer stem cell concept for pancreatic cancer and its use as a platform for the development of novel, more effective treatment modalities. Although we have conclusively demonstrated the exclusive tumorigenicity of isolated human pancreatic cancer stem cells and their inherent resistance to standard chemotherapy, the origin of these pancreatic CSCs and their involvement in

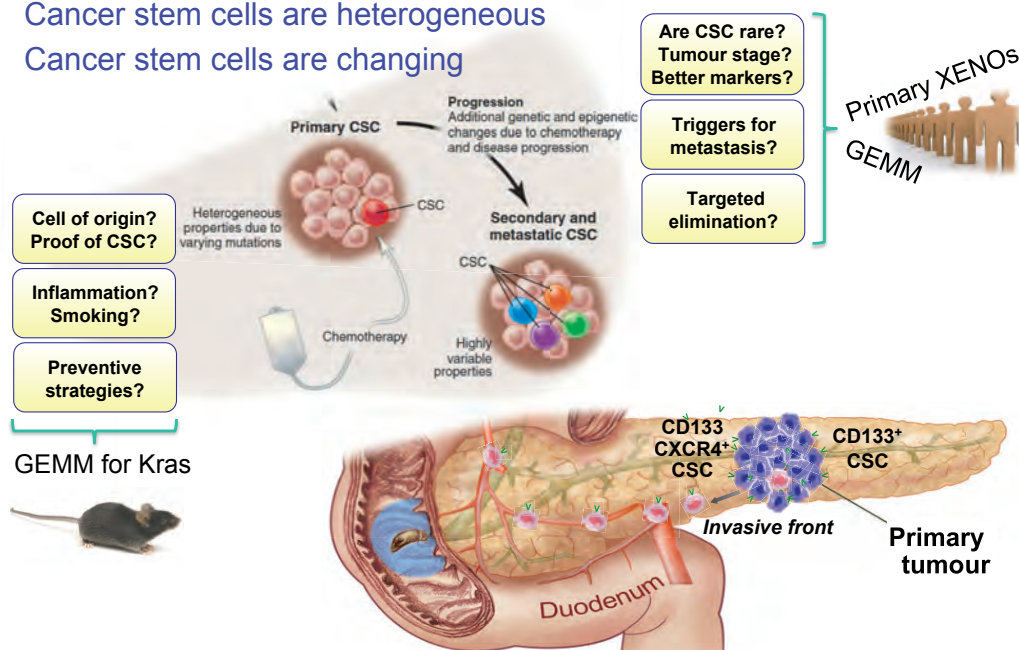
chronic inflammatory processes remain unclear (Figure 1). Most importantly, only the specific elimination of CSCs *in vivo* using stringent genetic tools will eventually confirm their crucial relevance in tumour promotion and will set the stage for the development of targeted therapies against these cells as the putative root of the tumour.

The cell-of-origin can be identified through genetic control of *Kras* expression in different cell populations *in vivo*. It is tempting to speculate that the precise cell-of-origin depends on the context in which the specific cell is captured, i.e. whether

### Studying pancreatic cancer stem cells in mouse & man

Cancer stem cells are heterogeneous

Cancer stem cells are changing



**Figure 1:** The cell of origin for cancer stem cells may be context-dependent and diverse cancer stem cell populations may evolve during progression. We have identified a subpopulation of migrating cancer stem cells. Current studies are addressing the cell-of-origin in the context of smoking and pancreatitis as well as their further evolution during tumour progression and therapy.

*Kras* mutations occur in the context of chronic inflammation or in association with regenerative processes. Chronic pancreatitis, especially its hereditary form, is a major risk factor for developing pancreatic cancer. Therefore, the model of pancreatitis-induced cancer is used to establish more precisely why this is the case. Our genetically tractable model systems for inflammation-associated cancer now make it possible to unravel these basic mechanisms. As we have already demonstrated the hierarchical nature of pancreatic cancer in these genetically engineered mouse models of pancreatic cancer, we are now using these models not only to investigate the specific origin of pancreatic CSCs but also the potential promotional role of chronic pancreatitis on the initiation of their transformation. Most importantly, taking advantage of a suicide gene approach, we will then be able to conclusively demonstrate that the elimination of a specific stem cell population in the regenerating pancreas abrogates the progression of early lesions to invasive ductal adenocarcinoma.

*Comprehensive cancer stem cell profiling.* This is a key aim for several ongoing projects in our laboratory. This technology is used to elucidate whether and how molecular mechanisms involved in the tight homeostatic regulation of the normal stem cell compartment, once subverted, are also implicated in tumourigenesis, progression, metastasis, resistance to therapies and disease relapse. Moreover, we are deciphering the specific and derailed regulatory machinery of CSCs *in vitro* and *in vivo* that determines their self-renewal capacity, their unbalanced ratio of symmetric and asymmetric cell divisions and their differentiation capacity as putative starting points for therapeutic interventions.

Specifically, we are analysing their regulatory machinery to:

- investigate the epigenetic profile of differentiated (cancer) cells vs. progenitors vs. normal stem cells vs. embryonic stem cells, to characterise critical epigenetic patterns;
- study epigenetic patterns of CSCs compared to those of normal stem cells to identify specific molecular signatures that

distinguish stem cells from committed precursors (normal "stemness" signature) and normal from cancer stem cells (cancer "stemness" signature);

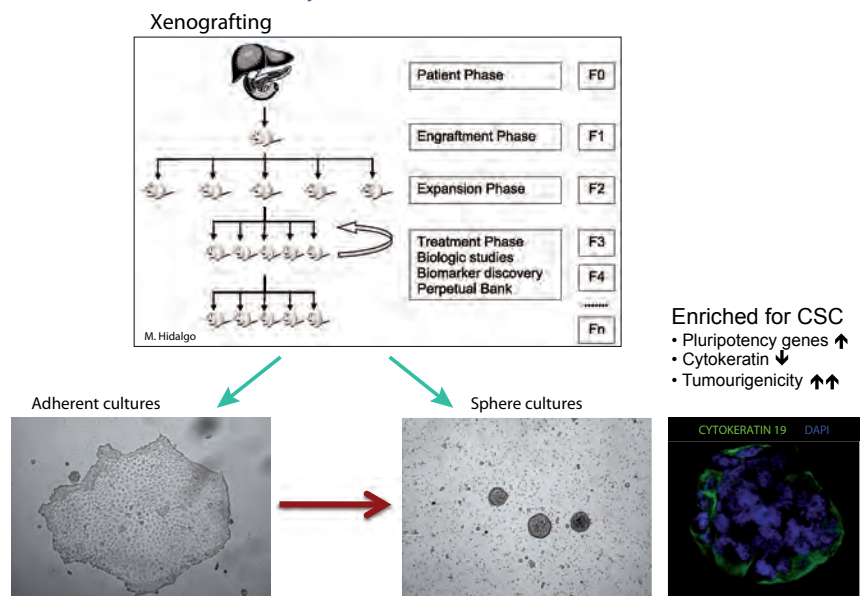
- identify new pathways that determine and control CSCs using *in silico* network biology;
- define the genetic conditions that permit progenitors (or even differentiated [cancer] cells) to transform into cancer stem cells (induced CSCs);
- study epigenetic patterns of cancer stem cells compared to those of migrating/circulating CSCs to identify specific molecular signatures that distinguish CSCs from metastasis mediating cancer stem cells ("MET" signature);
- identify the cancer stem cell niche and cell signals driving induction, maintenance and drug resistance of CSCs.

## Development of novel targeted therapies against cancer stem cells

To ultimately develop a targeted therapy for the elimination of CSCs as the root of the tumour we are pursuing screening analyses using cell culture conditions that strongly enrich for human CSC populations as compared to differentiation-promoting culture conditions (Figure 2). For these analyses, we are also focusing on the demonstrated resistance of CSCs to chemotherapy and tumour-initiating capacity. The subsequent functional characterisation of newly identified genes

**Figure 2:** We have developed a comprehensive set of models for the *in vitro* and *in vivo* characterisation of pancreatic cancer stem cells that are used for the identification of novel therapeutic modalities.

## *In vivo* & *in vitro* systems for cancer stem cells



and their biological function concerning resistance to hypoxia, tumour invasiveness, and metastasis in pancreatic cancer should then provide us with new cognitions about the role of cancer stem cells in cancer biology. These investigations will be followed by preclinical and clinical studies aimed at identifying potential active agents. We will take advantage of our newly established pancreas cancer xenograft model to isolate pancreatic CSCs and a syngeneic model of genetically engineered mice with pancreatic cancer.

Using this technology, we have already identified several targets that, preferably in combination, are capable of eliminating pancreatic cancer stem cells. Specifically, *ex vivo* pre-treatment of pancreatic cancer cells with the naturally occurring Shh inhibitor cyclopamine showed a decline in CSC content, surprisingly, this did not translate into reduced tumourigenic activity of pancreatic CSCs in a single-agent therapy. Interestingly, however, cyclopamine alone significantly decreased the metastatic activity of the treated cells as compared to treatment with gemcitabine alone. The simultaneous application of cyclopamine plus gemcitabine interestingly completely eliminated the CD133+ CXCR4+ migrating CSC population, which has been demonstrated to be exclusively responsible for the metastatic spread of pancreatic cancer.

Since Shh inhibition alone was not able to completely eliminate the CSC population, we needed to identify additional targets. We showed that CD133+ cells in pancreatic cancers exhibit particularly high activity for mTOR signalling and that single-agent therapy with the mTOR inhibitor rapamycin caused a significant decrease in CD133+ CSCs. Inhibition of the mTOR pathway by rapamycin however was not sufficient to

eliminate CSCs completely. Intriguingly, only the combined inhibition of these two pathways by cyclopamine and rapamycin, together with gemcitabine, resulted in the desired targeting of CSCs. This triple (CRG) therapy resulted in a significant depletion of the pancreatic cancer stem cell pool. Implantation of cells that were pre-treated *ex vivo* demonstrated that tumourigenic activity was completely abrogated. In a more clinically relevant setting we investigated the effects of the triple therapy on patient-derived established pancreatic cancers. For the first time, we were able to show that a multimodal therapy that involves the inhibition of two relevant stem cell pathways in addition to chemotherapy represents a very promising approach, resulting in virtually complete elimination of CSCs, significantly reduced tumourigenic and metastatic activity, and long-term event-free survival.

Collectively, these and other ongoing studies in our laboratory will provide important clues as to how CSCs circumvent the physiological regulatory elements of stem cell functionality and, even more importantly, how these cells escape the response to standard cancer therapy. Our studies should lead to the discovery of more effective therapeutic strategies, some of which – such as the sonic hedgehog pathway – have already been discovered and are currently providing new therapeutic opportunities. In addition, the genomic characterisation of pancreatic CSCs will permit the implementation of functional genomics approaches to discover new therapies. Eventually, these new discoveries should allow us to develop novel, targeted and, most likely, multimodal treatment regimens for the successful elimination of these cells as the previously unrecognised root of the tumour.

## Publications

Saif J, Schwarz TM, Chau DY, Henstock J, Sami P, Leicht SF, Hermann PC, Alcala S, Mulero F, Shakesheff KM, Heeschen C, Aicher A (2010). Combination of injectable multiple growth factor-releasing scaffolds and cell therapy as an advanced modality to enhance tissue neovascularization. *Arterioscl Throm Vas* 30, 1897-1904.

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Lonardo E, Hermann PC, Heeschen C. (2010). Pancreatic cancer stem cells - update and future perspectives. *Mol Oncol* 4, 431-442.