ANNUAL REPORT 2012

ISREC FOUNDATION
A FOUNDATION SUPPORTING CANCER RESEARCH LINKING SCIENTISTS IN FUNDAMENTAL RESEARCH WITH CLINICIANS AND ENCOURAGING SCIENTIFIC TRAINING IN SWITZERLAND
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EDITORIAL
A LANDMARK YEAR

PREFACE FROM THE PRESIDENT OF THE FOUNDATION COUNCIL

Our Foundation was able again this year to increase the financial support granted to promote translational cancer research, scientific training and education.

The AGORA - Cancer Center project is rapidly taking shape. Developed and managed by the ISREC Foundation and supported by our partner institutions (CHUV, EPFL, UNIL and the Ludwig Institute), this project has become our main priority. The successful conclusion of the competition to choose the architects marks a concrete step towards the construction of the building. The firm of Behnisch Architekten in Stuttgart was chosen as the winner by the jury of experts.

The AGORA - Cancer Center will serve as a hub in Switzerland to combine basic and clinical research and become a Mecca for translational oncology. It will host up to four hundred researchers and clinicians. Stimulated by an atmosphere specifically aimed at promoting dialogue, they will have the task of providing solutions to the many challenges still posed by cancer, through discussing the issues and exchanging and arguing their different points of view.

Scientific education has also benefited from our support. Grants were awarded to students of the UNIL/EPFL Summer Program for training courses in laboratories carrying out cancer research. Grants were also awarded to PhD students participating in the programs “Molecular Life Sciences” (EPFL) and “Cancer and Immunology” (UNIL). The work that these young people will perform as they prepare their theses will help toward understanding of the mechanisms of cancer cells and will allow the identification of new therapeutic targets, in particular for leukemia, melanoma, sarcoma and cancers of the brain, colon and breast.

For the Foundation and the Swiss Institute for Experimental Cancer Research, 2013 is a pivotal year, as in June we will begin our 50th year of activities in the service of research to overcome cancer, a disease that still today remains a major challenge for society.

In conclusion, I would like to thank you for your confidence and your support. Your commitment to our cause is of the utmost value and remains indispensable to the fulfillment of our projects.

Yves J. Paternot
CANCER RESEARCH

CANCER FACTS AND FIGURES

There exist more than a hundred types of cancer as all the tissues of an organism can be affected and for certain tissues, several types of cancer are possible. Cancer is the 2nd cause of mortality in Switzerland, after cardio-vascular disease.

In Switzerland, about 37'000 new cases are registered each year (estimate of the National Institute for Cancer Epidemiology and Registration – NICER, 2012). More than 100’000 people live with a cancer diagnosed since less than 5 years (prevalence). (Source: Globocan 2002).

Today in Switzerland, four out of ten people (one man out of two and one woman out of three approx.) develop cancer during their life time, and unfortunately the disease can be cured in only half of the cases.

The risk of having a cancer before 70 years old is approximately 25% for men and 20% for women (Sources: FSO, NICER, 2012).

For all types of cancers, relative survival after 5 years is estimated in Switzerland at 48% for men and 57% for women (Source: EUROCARE 4; based on data of 7 cantonal registers).

VERY ENCOURAGING RESULTS

Even though the number of cancer cases increased during the last two decades (in particular because of early diagnosis and the ageing of the population), there has been a noticeable decrease in death rates for cancer overall (- 28.5% between 1991 and 2010).

In women, the most frequent cancer (responsible for death in 2010) is breast cancer followed by lung cancer and then by colon and rectum cancer. At diagnosis (incidence 2005-9) 1) breast, 2) colon and rectum, 3) lung, 4) melanoma. (Sources: FSO, NICER, 2012).

In men, the most frequent cancer (responsible for death in 2010) is lung cancer, followed by prostate cancer and then by colon and rectum cancer. At diagnosis (incidence 2005-9) 1) prostate, 2) lung, 3) colon and rectum and 4) melanoma. (Sources: FSO, NICER, 2012).

Several of the most frequent cancer types have regressed in Switzerland since end of the 1980’s. Among these types of tumors are the colon and rectum as well as the stomach in both sexes (these are cancer types which are related in particular to lifestyle) and female breast cancer, which has decreased thanks to clearly improved therapies and early detection.

It has however to be noted that lung cancer has notably increased among women, consequence of the rising number of smokers in young generations whereas it decreased by men.
CANCER RESEARCH

Although cancer mortality is decreasing, the disease is unlikely to disappear completely. Therefore the objective in the long term is rather to transform it into a chronic disease, which it will be possible to control and/or cure.

### Evolution of cancer death in Switzerland (1991-2010)

<table>
<thead>
<tr>
<th></th>
<th>Deaths 2010</th>
<th>Age-standardized rates*/ 100'000 inhabitants</th>
<th>Difference (%) 1991-2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cancers</td>
<td>16278</td>
<td>-28.5</td>
<td></td>
</tr>
<tr>
<td>Lung, bronchi (females)</td>
<td>1084</td>
<td>-8.0</td>
<td>78.1</td>
</tr>
<tr>
<td>Liver, bile duct</td>
<td>604</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>1179</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>461</td>
<td>-8.0</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>436</td>
<td>-11.4</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>312</td>
<td>-21.9</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>310</td>
<td>-22.9</td>
<td></td>
</tr>
<tr>
<td>Uterus corpus, ovary, adnexa</td>
<td>665</td>
<td>-26.1</td>
<td></td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>1686</td>
<td>-31.9</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>546</td>
<td>-35.4</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>1421</td>
<td>-35.6</td>
<td></td>
</tr>
<tr>
<td>Breast (females)</td>
<td>1411</td>
<td>-36.6</td>
<td></td>
</tr>
<tr>
<td>Lung, bronchi (males)</td>
<td>2059</td>
<td>-37.3</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>481</td>
<td>-56.8</td>
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<tr>
<td>Hodgkin’s disease</td>
<td>33</td>
<td>-62.5</td>
<td></td>
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<tr>
<td>Larynx (males)</td>
<td>69</td>
<td>-63.2</td>
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<tr>
<td>Uterus, Cervix</td>
<td>63</td>
<td>-65.6</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>9</td>
<td>-71.4</td>
<td></td>
</tr>
</tbody>
</table>

* European standard population

Source: Swiss Federal Statistical Office, Neuchâtel
HIGHLIGHTS 2012

EVENT SUPPORTED BY THE ISREC FOUNDATION IN 2012

Cell and developmental Systems
August 21-25, 2012 – Arolla (Switzerland)
The participants of the Arolla 2012 workshop discussed the latest findings in cell and developmental biology. A broad range of topics were covered during the talks and the poster sessions, ranging from cell division and embryogenesis to genome stability and aging. As in previous editions, the unique multidisciplinary flavor of the Arolla Workshop generated fruitful interactions amongst participants, including between young researchers and established scientists.

EVENTS ORGANIZED IN FAVOUR OF THE ISREC FOUNDATION IN 2012

Quiz night, Le Mont-sur-Lausanne
Event organized on April 20, 2012 by a student of the International School of Lausanne to raise money for cancer research. Further to the quiz night, CHF 1’000.- could be donated to the Foundation.

10 km of Lausanne race
To put an end symbolically to her successful battle against cancer, Sandra participated on April 28, 2012 in the 10 km of Lausanne race. With the encouragement of two friends who ran with her, as well as all those who believed in her and sponsored her race, Sandra was able to raise and donate CHF 5’000.-.

School ball, Glarus
In June 2012, the 5th year students of the high school of Glarus have organized their traditional school ball and have donated 10% of their earnings. As unfortunately several of the students are indirectly affected by this theme and consequently also the others, they chose together to support our Foundation and could donate CHF 700.-.

AGO Trophy, Lonay
Forty volunteers prepared the second edition of this trophy in memory of their friend Agostino who died from cancer. Nearly 300 people came and participated in the various tournaments organized in Lonay on June 17, 2012. The success of this event made it possible for the organizers to give CHF 9’000.- to the ISREC Foundation.

"Corcelles-le-Jorat“ Motorbike race
Since 1998, the committee of Team Girard has been bringing together owners, riders and lovers of old motorbikes, organizing every year an event for old-timers and donating half of the profits to the ISREC Foundation. After the fifteenth edition of the running which was held on August 25-26, 2012 in Corcelles-le-Jorat, they could donate CHF 1’000.-.
SUMMER RESEARCH PROGRAM
This year again, the ISREC foundation supported during 8 weeks (from July 9 to August 31, 2012) the training course in laboratories doing cancer research of six students from the EPFL and five students from the UNIL/CHUV. This first contact with a research environment represents for these young biologists or doctors a very enriching experience and an opportunity of creating new links on an international level. At the end of this program the occasion was given to them to present their work during a mini symposium which was organized on the UNIL campus on August 30, 2012.
SRP – TOPICS COVERED

Hannah Drummond Davico de Barros
**Group Prof. Daniel Constam** – EPFL/SV/ISREC
Molecular mechanisms of cyst formation and fetuin-A expression in Bicc1^{-/-} kidneys

Jennifer Kwan
**Group Prof. Cathrin Brisken** – EPFL/SV/ISREC
Mammary gland morphology of the ADAMTS18 knockout mouse model

Norbert Majubu
**Group Prof. Pierre Gönczy** – EPFL/SV/ISREC
Revealing centriole architecture in *Triconympha*

Nicola Mitwasi
**Group Prof. Yann Barrandon** – EPFL/SV/IBI
The impact of temperature on keratinocyte stem cells

Aleksandra Vancevska
**Group Prof. Joachim Lingner** – EPFL/SV/ISREC
Characterization of ubiquitination-deficient TPP1 mutants

Allen Zhu
**Group Prof. Jeffrey Hubbell** - EPFL/SV/IBI
Fibrin biomaterials: engineering hydrogel stability

Malak Benslimane
**Group Prof. Fabio Martinon** – UNIL/Department of biochemistry
The endoplasmic reticulum stress response in diffuse large B cell lymphoma

Akash Boda
**Group Dr Liliane Michalik** - UNIL/CIG
miR-21*: An important player in skin’s response to UV exposure

Hong Huat Hoh
**Group Prof. Gian-Paolo Dotto** – UNIL/Department of biochemistry
Racial differences in susceptibility to skin cancer: genetic polymorphisms in the ATF3 and AP-1 network genes

Jelena Tosic
**Group Prof. Winship Herr** – UNIL/CIG
Effects of post-translational modifications on OGT activity
*OGT* = *O*-linked *N*-acetylglucosamine transferase

Zuzanna Urban
**Group Prof. Nicolas Mermod** – UNIL/Institute of biotechnology
The role of DNA recombination in cell cycle progression
"ALLOCATED GRANTS"
The “allocated grants” are awarded to the best PhD students taking part in doctoral programs in biology or medicine. The ISREC Foundation receives a donation from a physical or moral person and is guarantor of the use of the full amount for the specific project. The Foundation controls the management of the grant.

GRANT “RICHARD AND RITA BARMÉ”
Function of telomeres and their molecular composition

This "allocated grant" amounting to CHF 80’000.- per year was awarded to Larissa Grolimund in October 2008 for 48 months. Larissa Grolimund is working in the group of Prof. Joachim Lingner, EPFL/SV/ISREC.

Project description
Telomeres protect the linear ends of eukaryotic chromosomes by preventing chromosomal end-to-end fusions and telomere attrition. They consist of repetitive DNA sequences, telomeric repeat containing RNA (TERRA), and proteins. Telomeres play a crucial role in chromosome stability and cancer biology. With each cell division, telomeres shorten, as the replication machinery of the cell is not capable to fully copy the very terminal part of chromosomes. Therefore, after a certain number of cell divisions, telomeres get critically short and elicit signals in order to stop the proliferation. A cell can circumvent the signal for critically short telomeres by activating mechanisms that elongate telomeres. Mostly, telomere elongation is effected by the expression of the telomerase enzyme. In this case, cells gain the ability to divide indefinitely, which can eventually lead to the formation of cancer.

In our laboratory, we are focusing on the identification of molecular mechanisms that regulate and control telomere length and function in normal cells and cancer cells. We aim at developing a new methodology that allows the identification of proteins that are present at telomeres. Importantly, the technique should allow us to determine variations between protein compositions of telomeres from different cells such as normal cells and tumor cells. In this context, the study will provide useful information to understand the role and regulation of telomeres in normal and, more importantly, in cancer cells. Prospective, the discovery of novel telomeric proteins may identify new targets for cancer therapies.

Until now, the complete molecular composition of telomeres has not been described and it has remained enigmatic how the telomeric protein composition (telosome) may change during various states such as for example during the cell cycle in order to regulate telomerase or upon telomere shortening to induce cellular senescence.
"...> ACADEMIC TRAINING

Results obtained during the third year of PhD
Quantitative Telosome Isolation Protocol (Q-TIP): Elucidation of telomere composition in various cell states

We established a quantitative telosome isolation protocol (q-TIP) that enables the determination of telomeric protein composition. In this method, chromatin is chemically cross linked and telosomes are purified with antibodies against TRF1 and TRF2, two telomere-specific factors. Q-TIP also involves the use of SILAC (stable isotope labeling of amino acids in cell culture) based mass spectrometry in order to compare in a quantitative manner the telosomes obtained from cells in different states. With q-TIP, we specifically enrich for telomeric DNA and associated proteins as confirmed by the detection of the telomere-specific shelterin complex and other known telosome components. We have applied q-TIP to cells with long and short telomeres and we can observe quantitative differences in the protein composition between these states. Moreover, we have discovered novel telomeric factors. Notably, for one of the newly isolated complexes, the transcription export complex (TREX), we have verified its presence at telomeres by complementary techniques. We are currently studying the telomere specific role of the newly identified telomeric proteins.

In summary, q-TIP enables the identification of novel telomeric proteins and the elucidation of protein composition variations at telomeres under different cellular conditions.

Figure legend
a) Workflow of Q-TIP. Two different cell lines are grown in either light or heavy SILAC medium for 8 population doublings. Cells deriving from the two different conditions are mixed in a 1:1 ratio, crosslinked, lysed and sonicated. Telosomes are immunoprecipitated with antibodies against known telomeric proteins (TRF1/TRF2). The enriched protein-DNA complexes are then washed and eluted. Following the crosslink reversal, the proteins are identified and quantified by mass spectrometry. Peptides deriving from the two conditions are distinguishable due to their mass differences. As a negative control we perform the experiment with non-specific IgG.

b) Non-exhaustive list of already known and novel telomeric proteins identified by q-TIP.

c) Scatter Plot representing the ratios between factors isolated from telosomes of short and long telomeres. Proteins present in the lower left quadrant represent factors that are significantly more abundant at long than at short telomeres, whereas proteins found in the middle of the graph show no variation between long and short telomeres.
GRANTS

"ISREC GRANTS"
The «ISREC grants» or financial supports from the ISREC Foundation for a thesis are awarded to the best PhD students taking part in doctoral programs in biology or medicine. These grants amounting to CHF 80'000.- per year are awarded for four years. They are financed by donations and legacies.

GRANT " MOLECULAR BIOLOGY OF CANCER AND INFECTION"
Identification of Hes1 target genes in murine and human T-ALL

This “ISREC grant” amounting to CHF 80’000.- per year was awarded to Silvia Wirth in September 2009 for four years. Silvia Wirth is working in the group of Prof. Freddy Radtke, EPFL/SV/ISREC.

Project Description
T cell acute lymphoblastic leukemia (T-ALL) is the most common hematopoietic malignancy in children. Improved chemotherapy cures 80% of T-ALL patients. However, patients who relapse have poor prognosis. It is therefore important to understand the molecular pathways that control disease development to improve treatment of relapse patients. A chromosomal translocation was identified 18 years ago in a small cohort of human T-ALL patients leading to the constitutive activation of the Notch1 signaling cascade. In 2004 a landmark study showed that the majority (>50%) of human T-ALL patients have small changes within the Notch receptors (so called point mutations), which leads to the aberrant activation of this signaling cascade and thus to cancer. This placed the Notch1 signaling pathway at the center of T-ALL pathogenesis.

Active Notch signaling in T cells leads to the expression of a certain number of genes, including the transcriptional repressor Hes1. We started to study the role of Hes1 in different mouse models, which develop T-ALL and thereby mimic the human disease. Preliminary results are very encouraging and show that Hes1 is essential for T-ALL development. Therefore, it is important to better understand and characterize Hes1 function on a molecular level. The emphasis of this project is to identify the genes that are negatively regulated by Hes1.

Results after 3 years
T-cell acute lymphoblastic leukemia (T-ALL) can be modeled in the mouse via expression of a truncated form of NOTCH1, Notch1 intracellular domain (NICD), either through expression from the ROSA26 locus, or via infection of hematopoietic stem cells with retroviruses expressing NICD. We distinguish an early and a late stage of T-cell leukemia in the retroviral model (1). Our experiments show that Hes1 has an impact on disease outcome only in the early, but not in the late stage of the retroviral leukemia model. We therefore set out to assess differences in gene expression in the presence and absence of Hes1 using RNA-seq on early stage tumor cells.
...> ACADEMIC TRAINING

We also investigated the function of HES1 in human T-ALL and could show that compared to cells expressing scrambled shRNA, the knockdown of HES1 with shRNA in two patient-derived cell lines (T-ALL1, CUTL-1) results in severe growth retardation accompanied by increased cell death. In order to identify genome-wide targets of HES1, we planned to apply ChIP-seq, a technique that combines chromatin immunoprecipitation (ChIP) with high-throughput (Illumina) DNA sequencing. We performed ChIP-seq in the human T-ALL cell lines T-ALL1 and CUTL-1. As it is known that HES1 negatively autoregulates itself (2) we used the HES1 promoter as a positive control locus (Figure). Currently, we are in the process of analysing the data. We aim to integrate the ChIP-seq and RNA-seq data sets to find conserved targets of HES1.


Figure legend
HES1 ChIP-seq read enrichment in the HES1 promoter region. (A, B) The fold enrichment relative to IgG was evaluated by qPCR at the HES1 promoter (HES1 PRO) and in the HES1 open reading frame (HES1 ORF) in CUTL-1 and T-ALL1 HES1 ChIP samples. (C) A screenshot from the USCS genome browser (human genome at chr3:193,850,788-193,856,820) shows the promoter region and the start of the HES1 gene. The black bar (PCR) indicates the region amplified by qPCR. ChIP-seq reads aligned to the reverse strand are shown in red, reads aligned to the forward strand are shown in blue.
GRANTS

GRANT «CANCER AND IMMUNOLOGY”
Role of mesenchymal Notch signaling in melanoma development and progression

This “ISREC grant” amounting to CHF 40'000.- per year was awarded to Elena Menietti in June 2011 for four years. Elena Menietti is working in the group of Prof. Gian-Paolo Dotto, Department of Biochemistry, UNIL.

Introduction
The aim of the project is to test whether alterations of cell-cell communication resulting from down-modulation of Notch signaling may play a role in skin cancer development.

The original proposal, as suggested by the title, was focused on melanoma. However, the role of Notch signaling in this context is poorly understood and for this reason we decided to switch our focus to squamous cell carcinoma, one of the most frequent types of solid human tumors, in which the tumor suppression function of Notch is now well established.

It has been shown that the tumor microenvironment exerts a huge effect on cancer onset and development, driving cancer research to a more complicated level, in which not only pathways within the cells are important, but also the relationship of these particular cells with the surrounding cells and the environment; for example, the tumor stroma has been found to host chronically activated fibroblasts, so called «cancer-associated fibroblasts» (CAFs) which, in contrast to normal fibroblasts, have a demonstrated ability to enhance tumorigenesis and/or invasiveness of cancer cells, forming an appropriate niche for cancer development. CAFs are capable of interacting with the tumor through the production of various kinds of diffusible factors, and maybe also by cell-cell contact interactions.

Our assumption is that normal stroma and epithelial cells can also interact with the tumor, eventually mitigating its aggressiveness. Notch signaling is very important for intercellular communication and it is highly context-dependent. It may act as a tumor suppressor, for example in keratinocytes, or as an oncogene, as is likely to be the case in melanocytes.

Some experiments have shown that in the mesenchymal compartment the loss of Notch signaling is capable of inducing a CAF phenotype.

Results after the first year
In this first year we initially performed some in vivo experiments, which showed that the injection of cancer cells with normal cells, both epithelial and mesenchymal, gives rise to less aggressive tumors.

Then, to investigate whether our reference pathways are involved, we developed a system to induce p53 and Notch signaling in cells.
We have performed several experiments of co-culture between cancer cells and the same cells in which we induced p53 or Notch signaling. Preliminary results show that the co-culture with cells in which p53 has been induced results in an increase in the proliferation of cancer cells, while the co-culture with cells in which Notch has been induced induces growth arrest in cancer cells. The induction of either p53 or Notch in the tumor cells induces growth arrest, but the effect on neighboring cells is strikingly different.

Our future aim is to understand the mechanisms by which the cross-talk between different cells works, investigating the possibility that microvesicles derived from a cell can convey information and «orders» to its neighbors.

The results achieved to date strongly encourage us to investigate how normal cells can be used as a unique help to regulate the aggressiveness of cancer cells.

Figure legend

A: Picture of cancer cells (red and green) cultured with cancer cells in which Notch has not been induced.
B: Picture of cancer cells (red and green) cultured with cancer cells in which Notch has been induced.
C: Quantification of red vs. green cells. Cells become red when they are growth-arrested, while they are green when they are proliferating.

Notch induction in cancer cells is capable of inducing growth arrest in neighboring cancer cells.
**GRANTS**

**GRANT «CANCER AND IMMUNOLOGY»**

The role of Notch in TH17 cells differentiation and its relevance in cancer

This "ISREC grant" amounting to CHF 40'000.- per year was awarded to Manuel Coutaz in June 2011 for four years. Manuel Coutaz is working in the group of Prof. Fabienne Tacchini-Cottier, Department of Biochemistry, UNIL.

**Specific aims**

Interleukin 17-producing helper T cells (TH17) cells have been identified as a distinct T helper (TH) cell lineage. There is evidence that in both humans and mice, TH17 cells play a pathogenic role in inflammation and in autoimmune diseases. However, their exact role in cancer is still controversial. Studies of TH17 cells have indicated either a tumor-promoting or anti-tumor role in different types of cancer. Several reports have recently shown that the Notch signaling pathway might be involved in the generation of TH17 cells. We propose here to investigate: 1) the role of Notch receptor signaling in TH17 cell differentiation and 2) the contribution of Notch receptor signaling on TH17 cells in the tumor microenvironment and in other in vivo models inducing a TH17 cell response.

**Background and significance after one year**

Distinct subsets of CD4⁺ TH cells express different cytokine profiles that determine their function. TH17 cells are characterized by the expression of the transcription factor RORgammaT and in addition, the secretion of the IL-17A, IL-17F, IL-21, and IL-22 cytokines. In several human cancers TH17 cells are reported in higher densities among tumor-infiltrating T lymphocytes. However, the role of these cells in the tumor microenvironment is still unclear. In patients with established epithelial cancer, the presence of TH17 cells correlates with reduced tumor progression and improved patient survival. TH17 cells are also induced in experimental mouse models of cancer. Adoptive transfer of TH17 cells shows their capacity to suppress tumor growth. In contrast TH17 cells that infiltrate experimental induced tumors appear to promote tumor growth. These distinct roles of TH17 cells in cancer can be related to their plasticity and their potential to secrete in some settings IFN-gamma, an important cytokine required for tumor eradication.

Notch is a cell surface receptor involved in intercellular communication, regulating cell lineage and differentiation fates in many organs and tissues. Notch signaling is initiated by the ligand engagement of the Notch receptor. There are four Notch receptors (N1-4) and five Notch ligands (delta-like (Dll) 1, 3, and 4; Jagged 1 and 2).

The biological outcome of Notch activation is highly context-dependent. This also applies to the control of cancer development.

The present project will define the role of Notch receptor signaling in TH17 cell differentiation. This will be first investigated with an in vitro approach. Then the role of Notch receptor signaling in TH17 cells within the tumor microenvironment will be analyzed in vivo using an experimental model of melanoma.
Figure legend
In the United States, malignant melanoma skin cancer is currently the sixth most common cancer in men and the seventh most common in women, with 70'230 new cases that were diagnosed and with 8'790 people that died of melanoma in 2011.
In mouse adoptive transfer model, TH17 cells have been reported to induce tumor regression.

Publications
> Auderset, F., Coutaz, M., Tacchini-Cottier, F.
The role of Notch in the differentiation of CD4⁺ T helper cells.
> Auderset, F., Schuster, S., Coutaz, M., Koch, U., Desgranges, F., Merck, E., MacDonald, H.R., Radtke, F., Tacchini-Cottier, F.
Redundant Notch1 and Notch2 signaling is necessary for IFNγ secretion by T helper 1 cells during infection with Leishmania major.
GRANTS

GRANT «CANCER AND IMMUNOLOGY"  
Crosstalk between T lymphocytes and melanoma cells

This "ISREC grant" amounting to CHF 40'000.- per year was awarded to Natalie Neubert in January 2012 for four years. Natalie Neubert is working in the group of Prof. Daniel Speiser, Clinical Tumor Biology & Immunotherapy Group, LICR@UNIL.

Introduction
In 2008, over 67'000 new melanoma cases and over 14'000 deaths due to this disease were reported in Europe, with the highest incidence in Switzerland. Despite considerable medical progress during the last few years, the prognosis of patients with metastatic melanoma remains poor. Cytotoxic CD8+ T cells are an important class of immune cells. In patients, they infiltrate the tumor microenvironment and can attack melanoma cells (Pittet et al., 1999; Romero et al., 1998; Zippelius et al., 2002). The tumor infiltration of CD8+ T cells is associated with good prognosis in various types of cancer including melanoma (Fridman et al., 2012). These observations provide a strong rationale to design specific immunotherapy interventions aiming at inducing and/or boosting potent tumor-specific cytotoxic T cell responses. However, although CD8+ T cells can destroy tumor cells, the anti-tumor immune response does not usually lead to complete melanoma eradication. Even under optimal conditions in in vitro cytotoxic killing assays, most cancer-specific CTLs do not kill 100% of the target cells (Rufer et al., 2003; Zippelius et al., 2004). Much research has focused on understanding and increasing the cytotoxicity of CTLs. A still unanswered question is what happens to the surviving tumor cells in humans. The goal of this project is to shed more light on the dialog between the tumor cell and the infiltrating cytotoxic T cells. A better understanding of the complex network of stimulatory and inhibitory interactions between tumor cells and host cells will likely reveal novel target molecules and open options for anti-cancer therapy.

Results after one year
Development of an experimental system to study cytotoxic T cell – tumor cell interactions
Tumors are complex, heterogeneous tissues including several different cell populations that interact with tumor cells. Despite the fact that some of these interactions may play key roles in pathogenesis, they still remain poorly understood. In this project, we chose a co-culture system of cytotoxic CD8+ T cells and melanoma cells to screen for important interactions between the two players. The identified molecules and pathways will then be studied in depth.

Generation of working material
We identified five melanoma patients of whom both tumor cell lines and tumor specific CD8+ T cells are available. For one additional patient, tumor cell lines, but no tumor specific CD8+ T cells are available. Two out of the six tumor cell lines have already been amplified and the remaining four are currently being expanded in culture to obtain large cell numbers required for our experiments.
Concerning the T cells of the sixth patient, we isolated 401 tumor-specific CD8 T cell clones from his blood using a FACS Sorter. 72 clones could be amplified, 56 of which still displayed CD8 and a tumor-specific T cell receptor after amplification. Out of 14 sufficiently amplified T cell clones, four could recognize and kill tumor cells in a functional test.

**Optimization of the co-culture system and experimental tools**

Various parameters of the culture system have to be optimized to allow reproducible and meaningful analysis of the T cell-tumor cell interaction. So far, we have determined how many tumor cells need to be seeded at the beginning of the experiment, which ratios of T cells : tumor cells must be further assessed, what will be the maximal duration of the co-culture, how to harvest the cells from the culture in order to get the maximal viable cell number, which markers to use in order to remove dead cells from the analyses and how to separate the T cells from the tumor cells for analysis after the co-culture.

In the second year of the project, we will complete the development of the co-culture system and start investigating the interactions between the T cells and the tumor cells at the gene expression and protein levels. Our hypothesis is that T cells may induce tumor cells to produce “malignancy factors”. Such factors may support the further expansion of tumors, for example by stimulating cells of the tumor microenvironment, by mobilizing novel (hematopoietic) cells involved in tumor growth, and/or by supporting pre-metastatic niches.

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**Figure legend**

(A-D) Development of a cell co-culture system to study T cell – tumor cell interactions.

(A) Isolation of tumor specific (tELA positive) CD8 T cells from patient blood using FACS sorting.

(B) Functional assay to assess isolated T cell clones for their ability to kill tumor cells. Tumor cell killing was measured by release of chromium 51 from lysed tumor cells into the medium.

(C) T cell and tumor cell counts after a three-day co-culture. Cells were seeded at the indicated T cell : tumor cell ratio. *For the 1:3 ratio, three wells were pooled for the cell count.

(D) Comparison of inflammatory cytokine production by T cells after a 3-day co-culture at different T cell : tumor cell ratios. Control 1 – T cells alone; control 2 – positive control: T cells activated using CD3/CD28 beads; control 3 – negative control, co-culture of T cells with non-target tumor cells (10:1 ratio); control 4 – same as control 3 but at a 1:1 ratio.
**Endoplasmic reticulum stress in cancer**

This "ISREC grant" amounting to CHF 40'000.- per year was awarded to Bojan Bujisic in January 2012 for four years. **Bojan Bujisic** is working in the group of Prof. Fabio Martinon, Department of Biochemistry, UNIL.

**Introduction**

The endoplasmic reticulum (ER) is an essential organelle that detects perturbation of cellular functions and restores homeostasis via the induction of the unfolded protein response (UPR). Hypoxia, nutrient deprivation and pH changes that are commonly present within tumor mass activate a range of cellular stress response pathways including the UPR. This response can trigger both pro-survival and pro-apoptotic signals. It is therefore essential to understand how modulation of the UPR alters the balance between these processes and contributes to carcinogenesis in different cell types. Moreover, the identification and use of drugs that direct the UPR in cancer cells towards promotion of cell death could lead to the development of new therapeutic strategies. Recently it has been suggested that HIV protease inhibitors, a family of drugs used to decrease viral replication in HIV patients, exert antitumor action by modulating a specific UPR response (Figure).

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**Figure legend**

**ER stress signaling**

Stress within the ER is detected by sensor proteins IRE1, PERK and ATF6, whose activation triggers three signaling cascades leading to an adaptation response that reduces the ER stress and restores homeostasis via the activation of the Unfolded Protein Response (UPR). Recently it was suggested that nelfinavir, an HIV protease inhibitor, could tip the balance of the UPR towards the promotion of apoptosis.

Deregulation of ER-signaling pathways was observed in different types of tumors. For example, gene expression data suggest that XBP1 target genes are upregulated in activated B-cell-like (ABC) lymphoma, a specific subset of diffuse large B cell lymphoma (DLBCL). The role of XBP1 and the UPR in this B cell malignancy found in around 40% of all lymphoma patients has not yet been investigated. My project is aimed at elucidating the role of UPR signaling pathways in tumors by focusing first on the role of XBP1 in DLBCL.
Results after the 1st year
To characterize the UPR response in DLBCL, I biochemically analyzed the response in tumor cell lines originated from the two main subsets of DLBCL: activated B-cell-like (ABC) and germinal center B-cell-like (GCB) DLBCL. I treated these cell lines with drugs that trigger ER stress such as tunicamycin and thapsigargin, or with the HIV protease inhibitor nelfinavir, and then monitored the activation of the UPR pathways. My results show that after induction of ER stress, ABC cell lines respond normally. These tumor cell lines induce the production of an active XBP1 protein and promote the upregulation of UPR-dependent genes, indicating that this response is functional in the ABC subtype of DLBCL. In contrast, we observed that upon stimulation with ER stress-inducing drugs, the GCB cell lines have an impaired response, characterized by a very weak induction of active XBP1. Given the dramatic differences in XBP1 activation between ABC and GCB DLBCL tumors, I analyzed the expression of IRE1, the ER-anchored kinase that regulates XBP1 activation. Consistent with my data on XBP1 activation, I found that GCB DLBCL cell lines show reduced levels of IRE1 compared to ABC cell lines. These preliminary data indicate that IRE1 deficiency is a hallmark of GCB DLBCL tumors and could contribute to increased sensitivity to ER stress-inducing drugs, possibly providing us with new prognostic tools and therapeutic strategies in DLBCL patients.

Future directions
To interrogate the physiological relevance of these findings, we designed two main strategies:
First, we will identify the role of XBP1 and IRE1 in the development of DLBCL by modulating the expression of the pathway components and analyzing the outcome in vitro and in mice.
Second, we will investigate whether deregulation of the UPR by therapeutics targeting the ER stress pathways such as nelfinavir could be exploited for the treatment of various DLBCL tumors, in particular tumors that display a defect in the XBP1-dependent adaptation response.
ISREC CHAIRS

To reinforce its support and to promote the advance of translational oncology, the Foundation decided to create three «ISREC chairs». These are created to give the possibility to young professors to start a career in research. Each chair is endowed with CHF 500'000.- per year for a period of six years and is financed by the fortune of the Foundation.

1ST ISREC CHAIR “TRANSLATIONAL ONCOLOGY”
Signaling mechanisms and novel treatment strategies for hematological malignancies

This chair endowed with CHF 500'000.- per year for a period of six years was allocated in March 2011. It is funded by the fortune of the ISREC Foundation. It was awarded to the research group of Prof. Oliver Hantschel (ISREC/EPFL).

Introduction
Leukemia is a cancer type that is characterized by the overproduction of certain types of white blood cells in the bone marrow and its premature release into the peripheral blood. Most leukemias are fatal if not treated readily after diagnosis. Over the past decades, several changes in the genetic material of leukemia patients, such as losses or duplication of certain genes, as well as swaps of genetic material between different chromosomes (so-called translocations) could be identified and linked to the pathophysiology of leukemia. In all cases, these genetic changes result in the expression of abnormal amounts or structurally altered proteins. Different technologies have been developed over the past 10 years that make it feasible to comprehensively study the genetic make-up as well as the proteins that are responsible for the altered signaling in cancer cells of individual patients. In parallel, engineered proteins and a growing number of specific drugs have been identified and can be used to attempt to better understand the abnormal signaling in leukemia cells. This can lead to the identification of additional ways by which tumor cells can be attacked with the hope that these new insights can be translated into useful therapies for cancer patients.

Project after one year and half
We are currently intensively studying the adapter protein Gab2 that is critical for the transmission of oncogenic signals in different leukemias. Gab2 serves as an assembly platform for proteins that mediate the activation of pathways leading to cell proliferation and inhibition of cell death, but the importance and contributions of individual pathway components and their hierarchy are not well understood, mainly because of a lack of selective inhibitors for individual signaling molecules. We are using small engineered proteins that are tailored to bind very specifically to individual signaling molecules and thereby prevent their activation. This approach will help us to understand which of the signaling pathways is critical for tumorigenesis and therefore worth to target. In addition to leukemias, the Gab2 pathways are also deregulated in other diseases, such as breast and lung cancer.
Many of the cancer causing proteins are enzymes that add phosphate groups to other proteins and thereby perpetuate the oncogenic signal. Several new drugs have entered the market in the past years that specifically block these enzymes and showed outstanding responses in cancer patients. The main drawback of these new drugs is that the targeted enzyme may adapt its structure and often becomes insensitive to the drug, so that the tumors start to grow again. Therefore, we are attempting to find alternative ways to inhibit these enzymes. One way is to identify regulatory binding sites that are distinct from the site where the available drugs are binding with the hope to be able to delay the development of resistance. With a second approach, we are trying to manipulate the activity of the proteins that are involved in regulating the enzymatic activity of the cancer-causing proteins. My laboratory has started its operation in March 2011 on the campus of EPFL, at ISREC, as part of the School of Life Sciences. I am very grateful that I was provided with excellent infrastructure and financial support to hire an international and interdisciplinary team of young, motivated and talented individuals. The laboratory currently consists of one postdoctoral researcher, three PhD students, one lab manager and a master’s student. Since I started, I have contributed to teaching activities at EPFL, UNIL and CHUV to train Lausanne’s next generation of cancer researchers and I am looking forward to further collaborations within the striving and dynamic Lausanne cancer research community.

**Figure legend**
On the left, the molecular structure of part of the leukemia-causing enzyme Bcr-Abl is shown. Different drugs that are able to block the cellular action of Bcr-Abl are shown on the right side. The red balls indicate possible changes in the structure of Bcr-Abl that lead to the insensitivity for the drug.
FUNDS

FUND "TRANSLATIONAL RESEARCH - STEM CELLS"
Identification of novel anti-cancer strategies

This "allocated fund" from a private donator and amounting to CHF 3.5 millions was allocated in 2005. It was awarded to the research groups of Prof. Michel Aguet (EPFL/SV/ISREC) and Prof. Ivan Stamenkovic (UNIL/CHUV).

Introduction
Phenotypic heterogeneity is characteristic of many tumors and may be explained by various mechanisms, which are not mutually exclusive. Typically, cells, which have acquired genetic alterations providing them with growth or survival advantages may outgrow and form subclones within the tumor bulk. Heterogeneity may also be caused by signals emanating from the tumor microenvironment, for example through inflammatory processes at the interface between tumor and surrounding tissues, which may promote migratory and invasive properties of tumor cells. Additionally, tumor heterogeneity may result from hierarchical differentiation processes, which govern the renewal of normal organs and tissues such as the hematopoietic system, the skin or the gastrointestinal epithelium. Residual hierarchical organization can typically be observed in some hematopoietic malignancies, but also in many solid tumors, whereby cancer stem cells (CSCs), which are capable of self-renewal give rise to progeny that is more differentiated, has lost pluripotent traits and may be less relevant for tumor maintenance and recurrence. Consistent with this CSC model are numerous reports suggesting that undifferentiated cells are primarily responsible for tumor growth and disease progression. Altogether, these observations suggest that in hierarchically differentiated tumors targeting undifferentiated stem-cell-like cancer cells, most probably in combination with agents targeting the tumor bulk, may improve therapeutic efficacy.

Both our groups have focused their work on investigating such CSCs in different models. Our work is increasingly inspired by the clinic and has in part reached the stage where potential drug candidates are being identified.

In 2012 the Stamenkovic group pursued elucidating mechanisms that lead to CSC emergence in Ewing's sarcoma family tumors (ESFT). One lead came from our earlier observations that microRNAs (miRNAs), and notably miRNA-145, are repressed in ESFT CSC, and that their overexpression results in the loss of CSC properties. To address the role of miRNAs in CSC development further, we performed miRNA expression profiles on CSC and non-CSC ESFT cell populations. CSCs were observed to display suppression of a broad range of miRNAs compared to non-CSCs. Because each miRNA can regulate the expression of numerous genes, this downregulation of a broad panel of miRNAs could have a highly significant impact on the gene expression profile of CSCs and govern their biological properties.

CSC give rise to progeny that has lost CSC features, suggesting that the mechanism which suppresses miRNAs involved in CSC maintenance must be reversible. We were able to identify partial suppression of the gene that encodes TARBP2, a protein implicated in miRNA maturation, in CSCs but not in non-CSC ESFT populations.
TARBP2 was indeed responsible for the maturation of a wide range of miRNAs in CSCs and its partial suppression explained the downregulation of their mature forms. Restoration of TARBP2 in ESFT CSC abolished their self-renewal and tumor-initiating properties, suggesting that therapeutic TARBP2 targeting could be a valuable therapeutic option to eliminate CSC. A recent study showed that RNA interference could be augmented by enoxacin, an antibiotic that enhances TARBP2 activity. We therefore assessed the potential applicability of enoxacin in ESFT treatment. Our results indicated that enoxacin depletes CSC subpopulations in ESFT in vitro and in vivo and offers an unexpected therapeutic reagent for ESFT. Ongoing studies are addressing the effect of enoxacin in combination with conventional therapies aimed at eliminating the tumor bulk. Preliminary results are highly encouraging and are hoped to launch new clinical trials for ESFT.

In a separate study, we have addressed the energy requirements and production in CSC. Using glioblastoma (GBM) as a model, we identified an RNA-binding protein, Imp2, that is overexpressed in GBM and that binds not only numerous RNAs that encode proteins associated with mitochondrial respiration but respiratory complex I proteins as well. Moreover, we discovered that Imp2 is required for respiratory complex I function and that it helps maintain oxidative phosphorylation (OXPHOS) in CSC. Finally, we were able to show that contrary to the tumor bulk, CSC are exquisitely dependent on OXPHOS for their survival and function, offering another potential means to eliminate them.

Publications
De Vito, C. et al., Cancer Cell, 21, 807-821 (2012).

The Aguet group pursued validating the interaction of BCL9 proteins with their binding partner beta-catenin as a potential therapeutic target in colon cancer with a view to attenuate metastatic dissemination and enhance susceptibility to chemotherapy. We have previously shown that genetic inactivation of this interaction in a mouse model of colon cancer resulted in abrogation of stem cell and other malignancy traits including metastatic propensity. We have now extended these studies to human colon cancer cell lines and confirmed that inhibiting the interaction of BCL9 proteins with beta-catenin through expression of a functionally inactive BCL9 protein leads to a reduction of stem cell traits, accompanied by enhanced cell differentiation (see Figure). Using the same model, others had described a striking correlation between expression of stem cell traits and resistance to chemotherapeutic agents. We will therefore assess to what extent inhibiting the BCL9/beta-catenin interaction in this human model enhances susceptibility to chemotherapy. Stimulated by our observations we had started establishing an assay for the identification of small molecular compounds capable of blocking the BCL9/beta-catenin interaction. Encouraged by the identification of first hit compounds, this assay has now been optimized for high throughput screening of large libraries of drug-like compounds, which will be carried out in cooperation with academic and industrial drug discovery platforms and form the main focus of our future work.
FUNDS

Publications

Figure legend
Human colon cancer cells (SW480) stained for surface expression of E-cadherin (green fluorescence). E-cadherin is a differentiation marker of epithelial cells whose expression correlates with attenuated malignancy, including reduced metastatic propensity.
Left panel: Parental SW480 colon cancer cells are composed of a majority of E-cadherin-negative cells; only islets of cells stain positive for E-cadherin.
Right panel: Inhibition of the BCL9/beta-catenin interaction through expression of a functionally inactive BCL9 protein promotes differentiation of SW480 colon cancer cells, as evidenced by the expression of the differentiation marker E-cadherin in the vast majority of cells.
Funds

Fund "Translational Research - Glioblastoma"
Embryonic stem cells for the modelization of brain tumors

This "allocated fund" from a private donator and amounting to CHF 350’000.-- was allocated to Dr. Olivier Preynat-Seauve in June 2011 for 3 years. It was awarded to Dr. Olivier Preynat-Seauve (laboratory for immunohematology, University Hospital of Geneva).

Introduction
Glioblastoma is a brain tumor associated with a very severe prognosis. To understand the biology of these tumors and discover novel therapeutic strategies, modelization in laboratory is an important issue. Modelization consists in the ability to reproduce in vitro the in vivo situation in patients in order to study the disease. The best model currently used to study glioblastoma is the injection of human cancer cells in mouse brain. This model is not optimal because it is based on a human/mouse interaction which does not recapitulate the real in vivo situation in patients.

Description of the project
Based on the use of embryonic stem cells, we have recently developed a method to generate in vitro human brain-like tissue. Introduction of human glioblastoma cells within this tissue generates a tumor resembling to the in vivo situation in patients, thus providing an innovative tool to study glioblastoma exclusively in the human species. The goal of our project is to develop this model and use it for the understanding of the disease and also to find new therapeutic compounds. Recently, molecular analyses on this model suggested that viruses could be associated to the disease.

Experiments completed during the last year
We are currently studying the molecular interactions between the tumor and the host brain tissue, in order to understand the aggressiveness of glioblastomas. Recently, molecular analyses of this model suggested that viruses could be associated with the disease. Manipulations of the model have indeed shown that the physical association between the tumor and its host tissue generates a molecular response called “type I interferon response”. This response is usually induced after viral infection. As viral etiology in glioblastomas is poorly documented, we have decided to screen for the presence of known viruses in biopsies of glioblastoma patients. For this purpose, we have used the powerful method of next-generation ultra-deep sequencing of nucleic acids. We are currently analyzing the data resulting from this sequencing.

Planned experiments
- Follow-up of the data to identify viruses in glioblastomas.
- Introduction of a detection system to quantify the ratio between the tumor and healthy tissue. Adaptation to multiwell plates will enable the screening of compound libraries in order to find new drugs against glioblastoma.
<table>
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<th>Figure legend</th>
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<td>This figure represents a molecular analysis of genes in glioblastoma patients. Red boxes indicate a molecular response called &quot;type I interferon induction&quot; which is indicative of defense against viruses. The blue color indicates that these pathways are not activated. A significant number of patients harbor this molecular signature which could be suggestive of virus infection.</td>
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FUNDS

FUND "TRANSLATIONAL RESEARCH – CANCER IMMUNOTHERAPY"
“CD1d-antitumor antibody” bifunctional fusion molecules to redirect the innate and adaptive immune responses to the tumor site

This "allocated fund" from a private donator and amounting to CHF 310’000.- was allocated in June 2011 for two years.
It was awarded to the research group of Prof. Pedro Romero (LICR@UNIL).

Introduction
The present project aims to activate and redirect to the tumor site a particular population of T lymphocytes called iNKT cells. These cells are known to transactivate the innate and adaptive immune responses. The iNKT cells are activated by glycolipids presented by the CD1d monomorphic MHC I-like molecule, mainly expressed on antigen-presenting cells (APCs). Numerous pre-clinical studies as well as clinical trials have reported their anticancer activity.

Results
In the last report of 2011, we convincingly showed in vitro and in vivo that targeting tumors with recombinant CD1d-antitumor scFv fusion proteins efficiently redirected the human and mouse iNKT cell–mediated cytotoxicity against cancer cells expressing the target antigen. Recent studies have reported that subtle chemical modifications in the lipid structure of the alpha-galactosylceramide ligand loaded on the CD1d molecule could modulate iNKT cell activation. In collaboration with Professor Steven Porcelli at the Albert Einstein College of Medicine in New York, we have indeed demonstrated that antitumor effects could be greatly enhanced by optimizing the glycolipid ligand loaded on the CD1d-antitumor fusion protein (Fig. 1).
Compared to the KRN ligand used so far, the stronger antitumor effects obtained with the best selected ligand, DB03-4, correlated with the faster release of Th1 cytokines that occurred after each injection of the recombinant protein. The next step will be to crosslink the glycolipid analog on the CD1d-scFv fusion protein to insure that all the fusion protein injected in vivo remains loaded with the glycolipid and retains the capacity to redirect activated iNKT cells to the tumor site. Another strategy to improve the efficacy of the CD1d-mediated immunotherapy is to increase the frequency of iNKT cells, which are present in rather small numbers in human and mouse blood (<1% circulating mononuclear cells). In this regard, recent clinical trials have involved the adoptive cell transfer (ACT) of ex vivo expanded human iNKT cells, which indeed showed promising clinical effects when co-transferred with ex vivo expanded dendritic cells (DCs) pulsed with the glycolipid. At the pre-clinical level, we first tested the CD1d-mediated immunotherapy in NKT transgenic mice that have 5 to 10-fold more circulating iNKT cells than the wild type strain. In these mice, repeated injections of the sCD1d-anti-CEA fusion protein did not exhibit systemic toxicity, and the antitumor activity was clearly more efficient than in the parental strain (Fig. 2 as compared to Fig. 1 KRN), with the elimination of four out of six established tumor grafts expressing CEA (200 mm³ at treatment start). These data fully demonstrate the efficacy of our CD1d-mediated strategy to redirect the potent cytotoxicity of iNKT cells against tumors.
FUNDS

They also support the importance of adoptively transferring high numbers of iNKT cells in cancer patients. The use of recombinant CD1d-antitumor proteins in cancer patients would have the advantage of redirecting the transferred iNKT cells to the tumor site and would thus replace the need for co-transferring dendritic cells, thus greatly decreasing the invasiveness of such treatment. In order to come closer to a clinical situation, we are currently performing adoptive transfer of transgenic iNKT cells into wild type tumor-bearing mice followed by treatment with sCD1d-antitumor fusion proteins. The final aim, developed in parallel, is to combine the iNKT-CD1d immunotherapy with therapeutic cancer vaccination, in order to exploit the capacity of iNKT cells to transactivate the adaptive immune response in addition to their intrinsic tumor cytotoxicity and NK cell transactivation ability.

Figure 1
In vivo anti-tumor activity of sCD1d-anti-CEA fusion protein loaded with either KRN or DB03-4 glycolipid analogs. Mice were grafted on the flank with 7x10^5 MC38-CEA tumor cells injected subcutaneously. I.v injections of PBS (untreated), KRN/sCD1d-anti-CEA (40µg) or DB03-4/sCD1d-anti-CEA (40µg) were started at day 7 when all tumors were palpable, and were repeated every 3 to 4 days for a total of 5 injections. Tumors were measured every two days and results represent the kinetic of tumor growth (mm³) as the mean of 6 mice per group.

Figure 2
Treatment of NKT transgenic mice with sCD1d-anti-CEA fusion protein induces powerful anti-tumor effects. Mice grafted s.c. with 1x10^6 MC38-CEA tumor cells were treated at day 12 with PBS (untreated) or KRN/sCD1d-anti-CEA (40µg), and i.v. injections were repeated every 3 to 4 days for a total of 3 injections. Tumors were measured every two days and results represent the kinetic of tumor growth (mm³) as the mean of 6 mice per group.
FUNDS

FUND "TRANSLATIONAL RESEARCH - SARCOMA"

Immunity of gastrointestinal stromal tumor (GIST)
Collaboration between the CHUV, Lausanne and the IGR, Paris

This "allocated fund" from a private donator and amounting to CHF 200'000.- per year was allocated in January 2012 for 5 years.
Unit INSERM U1015 and Center of Clinical Investigations IGR/Curie
Director: Prof. Laurence Zitvogel / IGR - Institute Gustave Roussy

Summary

Recurrent or severe GISTs bearing imatinib-resistant mutations in the causal oncogenes remain a clear unmet medical need. We have reported the relevance of immune predictors, such as NKp30 splicing variants in the prognosis of GIST, a tumor model expressing NKp30 ligands, sensitive to NK cell attack and for which KIT tyrosine kinase inhibitors stimulate NK functions (Borg C. et al. J. Clin. Invest. 2004, Ménard C. et al. Cancer Res. 2009, Delahaye N. et al. Nat. Med. 2011). Patients (and normal individuals) harbor different patterns of transcription of these NKp30 isoforms, leading to differential NKp30-dependent functions (Th1-type versus IL-10 cytokine release) dictating overall survival. The NKp30 transcription profile represents an independent prognostic factor overruling the KIT mutational status in GIST in that Th1-like NKp30 transcription patterns benefit from Gleevec and have a prolonged progression-free survival while patients presenting with an IL-10-oriented NKp30 profile rapidly relapse despite therapy with Gleevec. Importantly, Gleevec therapy can promote tumor infiltration by NK cells, most prominently in Th1 patterns. Part of this differential NKp30 expression pattern resulted from a single nucleotide polymorphism in position 3790 of the 3'UTR region of NKp30. Genetically and epigenetically determined NKp30 status represents a valuable biomarker for the clinical outcome of GIST.

Project description

The ISREC Foundation grant will help us in the following directions:
1) Explore how DNA hypomethylating agents may control/switch NKp30 transcriptional profiling on NK cell lines and in individuals' NK cells harboring a profile NKp30c (immunosuppressive)
2) Analyze the molecular links between the oncogenic mutation at diagnosis and NK cell phenotypes in tumor beds
3) Identify the potential NK cell differentiation mechanisms accounting for intratumor NK cell accumulation in good prognosis GISTs.

This research will allow us:
1) to design accurate predictors of response to tyrosine kinase inhibitors
2) to optimize the clinical management of bad pronosis GISTs and
3) will culminate in launching novel immuno-oncological combination strategies.
FUNDS

Institute Gustave Roussy comprises four main departments (Sarcoma Committee headed by Dr A. Lecesne, and Center of Clinical Investigations co-directed by L. Zitvogel and O. Lantz, as well as a research unit U1015 INSERM directed by L. Zitvogel), one outstanding surgeon, Dr Sylvie Bonvalot and two collaborative pathologists (Dr Terrier and affiliated Dr Jean François Emile in charge of molecular profiling of KIT/PDGFRA mutations). Long lasting collaborations are also established with other GIST medical experts (Prof. J.Y. Blay, CLCC Lyon, Prof. J.M. Coindre, Bordeaux, and Dr N. Isembert, CLCC Dijon). Our General Director, Prof. Alexander Eggermont is a worldwide expert in surgery of sarcoma and has been working for decades at the frontiers between immunotherapy and oncology. We are confident that this grant will promote significant advances in the management of metastatic GIST patients.

Figure legend
GIST infiltrated with NK cells stained with anti-NKp46 antibodies, which are located in fibrous trabeculae prior to Glivec. Cytolytic granules can be observed in NKp46 positive cells (figure on the left).
**FUNDS**

**FUND "TRANSLATIONAL RESEARCH - SARCOMA"**  
**Mechanisms of sarcoma initiation and development**  
Collaboration between the CHUV, Lausanne and the IGR, Paris

This "allocated fund" from a private donator and amounting to CHF 300'000.- per year was allocated in January 2012 for 5 years.  
Research laboratory : Institute of Pathology, UNIL/CHUV, Lausanne  
Director : Prof. Ivan Stamenkovic

**Introduction**  
Sarcomas are malignant tumors of bone and soft tissues that comprise about 2% of all human malignancies but as much as 15% of pediatric cancers. Despite multimodal therapy, most sarcomas retain poor prognosis with a high metastatic proclivity. Part of the reason for this is that sarcoma biology is still poorly understood.

**Aims of the project**  
We have undertaken studies aimed at identifying the cell of origin of a variety of sarcomas with the goal of elucidating the oncogenic events that lead to primary cell transformation and subsequent development of full-fledged tumors with the ability to metastasize. We showed that bone marrow-derived mesenchymal stem cells (MSC) are cells of origin of Ewing’s sarcoma, the second most common bone malignancy of children and young adults, and of myxoid liposarcoma. However, there is increasing evidence that other sarcomas, including osteosarcoma and synovial sarcoma also originate in MSC subsets.

Our observations led us to address mechanisms whereby MSC become transformed to develop Ewing’s sarcoma. We found that the fusion gene, EWS-FLI1, that is characteristic of Ewing’s sarcoma and arises as a result of a specific chromosomal translocation, induces a series of epigenetic modifications in MSC that lead to transformation. These modifications include changes in chromatin structure which alter the expression of key genes that regulate cell survival and proliferation as well as changes in expression of small non-coding RNAs, known as microRNAs (miRNAs) that control the expression of entire networks of genes. We were able to show that modulation of the miRNA expression profile in MSC leads to the emergence of cancer stem cells (CSC) in Ewing’s sarcoma. Cancer stem cells are believed to constitute the driving force in most malignancies in that they have the ability to self-renew and to give rise to more differentiated cancer cell progeny that constitutes the tumor bulk. Because CSCs typically divide slowly, they are relatively unharmed by conventional anti-cancer therapies aimed at eliminating rapidly proliferating cells.

**Results after the first year**  
In 2012, we completed an important study that elucidates a mechanism whereby cancer stem cells (CSC) in Ewing’s sarcoma are formed and maintained. We assessed the non-coding microRNA profile of Ewing’s sarcoma CSCs and found that these cells harbor a distinct miRNA expression pattern, where a broad panel of miRNAs is suppressed.
We explored the possible reasons for this broad suppression of miRNAs and found that the defect lies in the late stages of miRNA maturation. Because CSCs give rise to more differentiated tumor cells that have lost the capacity to initiate tumor growth and that display normal miRNA expression, the defect in miRNA maturation must be reversible, which hints at therapeutic possibilities. We discovered that the protein TARBP2, which plays a central role in the maturation of a broad spectrum of miRNAs, is partially downregulated in Ewing’s sarcoma CSCs and that re-expression of TARBP2 restores miRNA expression, thereby inducing CSC differentiation with loss of self-renewal and tumor initiating capacity. Thus, a drug that could enhance TARBP2 activity may be able to eliminate CSCs. We sought for possible candidates and discovered that members of the fluoroquinolone antibiotic family have the ability to enhance TARBP2 activity.

We therefore tested the effect of enoxacin, the member of the family that has been reported to have the maximal effect on enhancing TARBP2 activity, on Ewing’s sarcoma CSCs. Enoxacin enhanced miRNA maturation in CSCs that resulted in re-expression of the suppressed miRNAs along with loss of CSC self-renewal and tumor initiating capacity. Accordingly, administration of enoxacin in vivo reduced the growth of primary Ewing’s sarcoma xenografts and, most importantly, eliminated the CD133+ fraction of cells that corresponds to CSCs.

These observations provide the first insight into an approach that could selectively eliminate CSCs by inducing their differentiation. This would de facto impair the driving force of tumors and render conventional therapies far more effective. Ongoing studies will assess the effect of combining standard of care chemotherapy and enoxacin to treat Ewing’s sarcoma xenografts, with the goal of introducing such a combination into clinical trials.

Publications
ORGANIZATION

Founded on June 18th 1964, the ISREC Foundation is a private non-profit foundation. The Foundation started its activity with the creation of the Swiss Institute for experimental cancer research. Today its mission is to select and support translational cancer research projects and so to help the transfer of knowledge and collaboration between fundamental research and clinical research. The goal of these innovative projects is to translate discoveries into results and to have a positive impact on the future treatment of human cancer.

The Foundation is composed of:

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The Foundation Council is the highest managing authority of the Foundation. It allocates resources, appoints its members as well as those of the Scientific Board of the Management and of the Financial Auditors. Moreover, it approves the annual budget and the Foundation accounts.

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ORGANIZATION

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The Management selects with the help of the Scientific Board the research projects to be supported and addresses its opinion to the Foundation Council. It develops and proposes a strategy of fundraising and assumes the tasks which are allotted by the regulations of the Foundation.

Mr. Jean-Marc Tissot, Director

THE FINANCIAL AUDITORS
The financial auditors, whose tasks are allotted by law, are nominated by the Foundation Council. They are elected for one year. The 2012 mandate was entrusted to Ernst & Young, Swiss Fiduciary and Audit Company recognized by the Swiss Institute of Certified Accountants and Tax Consultants.
FINANCES

RESOURCES
To enable the Foundation to work towards its goals, the following resources are available: legacies, gifts, donations, the product of its fortune and all other resources. On December 31st 2012, the fortune of the foundation amounted to 44 millions of francs.

THE ISREC FOUNDATION IN 2012

Total of subsidies remitted in 2012 CHF 2'842'885

Total in support of scientific academic training CHF 353'000
Grant «Richard et Rita Barmé» CHF 80'000
Grant «Molecular biology of cancer and infection» CHF 80'000
Grant «Cancer and immunology» CHF 40'000
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Grant «Cancer and immunology» CHF 40'000
11 Grants «International Summer Research Program» CHF 33'000

Total in support of translational cancer research CHF 1'507'746
ISREC Chair I «Translational oncology» CHF 488'000
Fund «Translational research - stem cells»
- Colon cancer research CHF 133'136
- Ewing sarcoma cancer research CHF 58'472
Fund «Translational research - glioblastoma» CHF 175'000
Fund «Translational res. – cancer immunotherapy» CHF 153'138
Fund “Translational research - sarcoma” (IGR) CHF 200'000
Fund “Translational research - sarcoma” (CHUV) CHF 300'000

Project AGORA –Cancer Center CHF 982'138

Total gifts, donations, legacies, external grants received in 2012 CHF 1'776'362
43 spontaneous gifts from private individuals CHF 307'315
12 gifts from companies, associations, foundations CHF 78'600
3 gifts for allocated grants / funds CHF 659'488
104 gifts in memory of deceased people CHF 10'274
7 legacies, successions CHF 720'685

Capital of the Foundation (Free funds) CHF33'580'844
Reserved capital (Limited allocation funds) CHF 9'854'530
Grants CHF 960'000
Funds CHF 870'530
ISREC chairs CHF 8'024'000
SUPPORT THE ISREC FOUNDATION

MAKE A DONATION

The financing of the ISREC Foundation projects is mostly assured by donations and legacies from people aware of, and keen to help, our cause. Your support is therefore essential to the pursuit of our mission: the support of cancer research projects and the training of young scientists in Switzerland.

You can support our mission in various ways:
> by a donation
> by the sponsoring of graduate students
> by the sponsoring of young professors affiliated to a Swiss university or Institute
> by the sponsoring of post-doctoral scientists for the development of projects of high competence at the national level.
> by a legacy.

Whether modest or more important, every donation counts and contributes to our mission.

THANK YOU FOR YOUR SUPPORT

ISREC Foundation
Route de la Corniche 4 - 1066 Epalinges s/Lausanne / CCP 10-3224-9 (IBAN CH55 0900 0000 1000 3224 9) or UBS, 1002 Lausanne (IBAN CH11 0024 3243 G020 3554 0) or BCV, 1001 Lausanne (IBAN CH03 0076 7000 U032 9261 3)

FISCAL DEDUCTIONS

> Taxes at the federal level: A deduction of up to 20% of net income is possible, as long as the payment amounts to minimum CHF 100.-.
> Taxes at the cantonal level: The information available on the Zewo foundation web pages (www.zewo.ch) is applicable.

ISREC FOUNDATION TAXATION

The ISREC Foundation is recognized as a non-profit institution of public utility. Therefore your donations are exonerated from communal, cantonal and federal taxes.
Since 1964, several donors have supported through their gifts, subsidies or legacies our cause and contributed to the progress of cancer research. We are very grateful and thank each one of them most warmly.

of more than one million francs

CONTRIBUTIONS BETWEEN CHF 100 000.– AND CHF 50 000.–

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BOOK OF DONORS > ACKNOWLEDGEMENTS

ISREC FOUNDATION ANNUAL REPORT 2012

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ACKNOWLEDGEMENTS

At the end of this year we address our deepest gratitude to all our generous donors without whom none of our projects could have been realized.

We also would like to specially thank Mrs. Aylin Niederberger, in charge of administration, Mrs. Claudine Ravussin, in charge of communication and fundraising as well as our ambassadors, Mr. Didier Grobet and Mr. Jürg Karle for their faithful commitment.

You all contributed to the development and success of our foundation. We are very grateful and thank you warmly.