

ANNUAL REPORT 2015

ISREC FOUNDATION

A FOUNDATION SUPPORTING CANCER RESEARCH,
WHICH UNITES BASIC SCIENTISTS AND CLINICIANS UNDER
THE ROOF OF THE AGORA – CANCER CENTER AND ENCOURAGES
TRANSLATIONAL RESEARCH AND THE TRAINING OF YOUNG
SCIENTISTS IN SWITZERLAND



CONTENT

Editorial	1-3
Preface from the President of the Foundation Council Tribute to Mister Yves J. Paternot	
Highlights	5
Events organized in favor of the ISREC Foundation	
AGORA – Cancer center	6-7
Project	
Projects supported	9
Summer Research Program	
Grants	10-25
Cancer and Immunology / Molecular Life Sciences	
Chairs	26-31
Translational Oncology / Fundamental Oncology	
Funds	32-40
Glioblastoma / Sarcoma / Cancer Immunotherapy / Fundamental research	
Organisation	41
Foundation Council / Scientific Board / Management / Financial auditors	
Finances	42
Support the ISREC Foundation	43
Make a donation / Fiscal deductions / Taxation	
Book of donors	44
Acknowledgements	

MESSAGE FROM THE PRESIDENT OF THE FOUNDATION COUNCIL

Dear donors, friends and partners,

2015 marked the beginning of the implementation of the AGORA project. The building permit was delivered in March and the canton of Vaud granted the Foundation surface rights for 75 years in June. The consortium consisting of the Steiner and Marti companies was entrusted with the construction of the building and the groundbreaking ceremony took place in September. The work is progressing at an accelerated pace, as the aim is to inaugurate the building at the beginning of 2018.

At the end of 2015, Mr. Yves Paternot, president since 2005, in poor health and having reached the end of his mandate, retired from his post. In recognition of his huge commitment to the fight against cancer, he was appointed honorary president by the Foundation. Very unfortunately, he passed away shortly afterwards, in February 2016. At the end of 2015 also, two members of the council, Mrs. Martine Brunschwig Graf and Mr. Jean-Luc Chenaux, wished to terminate their mandate. We thank them for their commitment to the Council during the past years. Starting in 2016, Mr. Yves Bonzon, Mr. Thomas Paulsen and Mr. Pierre-Marie Glauser joined the council of which I am now the president.

In 2015, the Foundation supported the following enterprises:

a PhD scholarship in the area of cancer immunology, awarded to Mr. Efe Erdes for 4 years;
internships for undergraduate students in cancer research laboratories (5 UNIL/CHUV and 7 EPFL students);
a partnership within the framework of the Life Sciences Symposium 2015 "From Cancer Genetics to Personalized Health", School of Life Sciences, EPFL.

The new developments in cancer research, be it in the field of personalized medicine, immunology or any other promising area, are continuously and closely watched by our Foundation which, within the scope of its mission, will continue to support researchers working in these domains. We are very grateful for your invaluable support.

THANK YOU to all of you who have faith in the commitment of the Foundation to the fight against cancer!

Catherine Labouchère



TRIBUTE TO MISTER YVES J. PATERNOT



Mr. Yves Paternot joined the ISREC Foundation as a member of the Council in 2003 and presided over this committee for 10 years, starting in 2005. Shortly before his death, he was appointed honorary president in recognition of all the work he invested in the Foundation.

Strongly committed to the battle against cancer, a disease that affected him personally, he not only dedicated himself to implementing the goals of the founder, Dr. Henri Isliker, whom he deeply admired, but also to planning for the future. When the ISREC Institute joined the EPFL in 2008, he strove to ensure that the proceeds resulting from the sale of the buildings to the canton of Vaud were in one way or another invested in public institutions, which is how the AGORA project came into being. Starting in 2018, this emblematic building will bring together close to 300 scientists dedicated to translational cancer research.

A visionary, Yves Paternot was able to convince private individuals as well as institutions that a strong commitment is necessary in order to be able to allocate the resources needed by several multitalented teams meeting on a daily basis to share their experiences. He was convinced that these gatherings would lead to new ideas.

Within the Foundation, he managed to create an “ISREC team”. He loved the mountains’ and oceans’ wide open spaces and was likewise able to instill an open mind and a dynamic productivity in this team, which, now reinforced, will continue to uphold his principles.

His memory will thus be honored and his work will be continued: the AGORA building will be completed and one of the auditoriums will carry his name as a sign of gratitude for all he has contributed to the battle against cancer. His spirit will live on and mark those who come after him.

Thank you Yves!

Catherine Labouchère
President

IMPORTANT EVENTS

EVENTS ORGANIZED IN FAVOUR OF THE ISREC FOUNDATION

AGO trophy, Lonay

Fifty volunteers contributed to the success of the fifth edition of this trophy in memory of their friend Agostino who died of cancer. Close to 400 people were present and took part in the different activities and tournaments organized in Lonay on June 22, 2014. The success of this event enabled the organizers to make a donation of CHF 9'000.-.

Motorcycle hill climb “Corcelles-le-Jorat”

Since 1998, the Club Team Girard that includes owners, riders and fans of vintage motorcycles organizes a hill climb event for old-timers each year and donates half of the profits to the ISREC Foundation. The eighteenth edition took place on August 30-31, 2014 in Corcelles-le-Jorat.

En faveur de :





ISREC
FONDATION | STIFTUNG | FOUNDATION

Venez passer une journée de détente...
...en faisant une bonne action

DIMANCHE 21 JUN 2015 dès 9h00

St-Prex, centre sportif du Vieux Moulin

- Tournoi de Football à 7
Matchs à 2 équipes cadets et des
filles et filles de moins de 12ans
- Frais d'inscription :
CHF 150.- / équipe
- Tournoi de Pétanque
Frais d'inscription :
CHF 40.- / équipe
- Concours de fléchettes et tirs au but
- Château gonflable pour les enfants
- Tombola
- Nombreux autres jeux
- Restauration sur place

Informations & inscriptions
sur le site : www.tropheeago.ch

Avec le soutien principal de :

Agence générale Pascal Eyer

Rue de la Gare 28
Case postale 124
1710 Nyon 1
Tél. 026 / 267 77 77
Fax 026 / 267 77 77

Avenue Edouard Rod 4
Case postale 1027
1200 Nyon 1
Tél. 026 / 267 77 77
Fax 026 / 267 77 78

Siège à Nyon
www.allianz-suisse.ch/pascal.eyer
Allianz

18^e édition
Team Girard
www.team-girard.info

COURSE DE CÔTE
Corcelles-le-Jorat

29 & 30 août 2015



Les bénéficiaires
de la manifestation:
La Fondation ISREC
La Cordée Fondation Renée Delafontaine

Programme CHF 5.-

AGORA – CANCER CENTER

A POINT OF CONVERGENCE BETWEEN BASIC AND CLINICAL RESEARCH

In 2003, the ISREC Foundation decided to dedicate a large portion of its resources to the construction of a leading infrastructure, the AGORA – Cancer Center, on the site of the hospital and in direct interaction with patients.

This building will bring together groups involved both in basic and clinical research, uniting approximately 300 to 350 scientists and clinicians working to understand cancer development mechanisms and to develop novel therapies.



© Behnisch Architekten, Stuttgart

This project fulfils the primary mission of the ISREC Foundation; that is to support avant-garde cancer research by offering young scientists and physicians the opportunity to train and to develop their talent in an environment on the cutting edge of innovation.

Thanks to its unique setting, the AGORA – Cancer Center will allow the actors involved in translational cancer research to work in close collaboration with the hospital, so as to offer the patients personalized care protocols while accelerating the diagnostics-therapy cycle.

AGORA – CANCER CENTER

REINFORCING SYNERGIES

This project also aims to bring the academic institutions of the Lake of Geneva area (the CHUV, the UNIL and the EPFL in Lausanne, the UNIGE and the HUG in Geneva) and the centers of expertise in the German-speaking part of Switzerland closer together.



© Behnisch Architekten, Stuttgart

IMPLEMENTATION OF THE BUILDING SCHEDULE

January 2013	Selection of the project by the ISREC Foundation following an international competition
July 14, 2014	Submission of the application for the building permit
March 19, 2015	Issuing of the building permit
March 31, 2015	Open call for offers for the construction of the building
Sept. 23, 2015	Awarding of the building contracts
Autumn 2015	Start of the construction work
End OF 2017	Commissioning of the AGORA building
March 2018	Approval of all laboratories

SUPPORTED PROJECTS

SUMMER PROGRAM FOR UNDERGRADUATES (SUR/SRP)

For the eighth year running, the ISREC Foundation has supported the internship of five UNIL/CHUV and seven EPFL students in cancer research laboratories. During eight weeks (from July 6 to August 27, 2015), the young biologists or physicians were given the chance to discover the world of research for the very first time; a rewarding experience and the opportunity to establish new contacts on an international level. At the end of the program, on August 27, 2015, the students were invited to present their work during a mini symposium held at the UNIL campus.



Photo: Combined EPFL and UNIL Summer Research Program participants at students' symposium 2015

TOPICS COVERED

JOSEPH BOWNESS SAMSON

Group Prof. Winship Herr – UNIL/CIG

How do THAP Proteins Interact with HCF-1?

LIZA DARROUS

Group Prof. Nicolas Mermod – UNIL/IBT

The Methylation Status of AP2a Targets in Breast Cancer

ELIF KOCAK

Group Prof. Yann Barrandon – UNIL/CHUV

Thymoma's Incredible Talent to Stay Local

MIGUEL MARIN VERMELHO

Group Prof. Vladimir Katanaev – UNIL/DPT

Generation of a Stably Transfected HeLa Reporter Cell Line Expressing Inducible Fzd7 for High Throughput Screening

KRUTI VORA

Group Prof. Tatiana Petrova – UNIL/DEO

Inhibition of TGF- β Signaling Pathway to Prevent Downregulation of Prox1 in Lymph Node-Isolated Lymphatic Endothelial Cells

MATTEO CARTIGLIA

Group Prof. Jeffrey A. Hubbell – EPFL/SV/IBI

Selected Antibody Removal from Circulation: Application in Protein Replacement Therapies

INDRASHIS DATTA

Group Prof. Joachim Lingner – EPFL/SV/ISREC

Generation of CRISPR/Cas9 constructs for a CTC1 conditional KO cell line

VANESSA GERALDINE

Group Prof. Yann Barrandon – EPFL/SV/IBI

Exploring the Development of Merkel Cells in Whisker Follicles

GENEVIEVE M. GERHARD

Group Prof. Daniel Constam – EPFL/SV/ISREC

Study of the Interaction Between Bicc1 and 3' UTR of Adenylate Cyclase 6 mRNA Using Yeast Three-Hybrid System

IVAN ISTOMIN

Group Prof. Pierre Gönczy – EPFL/SV/ISREC

Role of Kinesin-5 in Centrosome Separation in *C. elegans*

MAGDALENA PLOTczyk

Group Prof. Olaia Naveiras – EPFL/SV/IBI

Testing Nicotinamide Riboside (NR) as an Accelerator of Hematopoietic Recovery after BM Transplantation

MARGARET WALKER

Group Prof. Viesturs Simanis – EPFL/SV/ISREC

Does the Cytokinesis Regulator PTPA Affect SIN Protein Localization?

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).

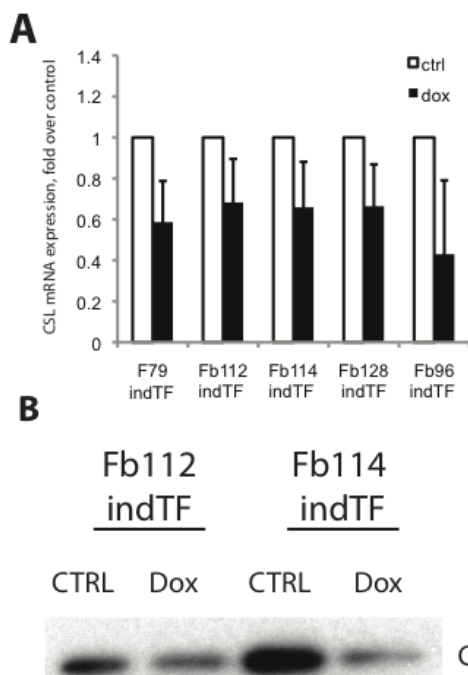
Introduction

CSL is a key transcription factor (TF), mostly acting as a repressor, which has been shown to have a highly context-dependent function. We have recently shown that CSL is important in maintaining skin homeostasis, as its specific deletion in mouse dermal fibroblasts creates an inducing environment for squamous cell carcinoma (SCC) development, possibly due to the conversion of dermal fibroblasts into cancer-associated fibroblasts (CAFs). Despite the wide interest in CSL as a transcriptional regulator, elucidating the mechanism of its own regulation has so far been neglected.

Understanding the regulation of CSL in fibroblasts is particularly important as this knowledge may help us find a way to recover CSL expression in CAFs (which, as we have demonstrated earlier, can be induced by CSL loss), in order to act on the microenvironment to limit tumor growth. Our goal was thus to understand the actors in CSL regulation.

The aim of the project was:

- 1) to understand the pathways which can regulate CSL;
- 2) to link these pathways to the response to UVA irradiation and pro-fibrotic signaling;
- 3) to understand whether differences in CSL regulation may be related to the differences in squamous cell carcinoma occurrence in different human populations.



(A) Fibroblasts from different individuals with overexpression of the transcription factor of interest show downregulation of CSL at the RNA level.

(B) Fibroblasts from different individuals with overexpression of the transcription factor of interest show downregulation of CSL at the protein level.

Results

To answer these questions, we started our investigation by looking at all the single nucleotide polymorphisms (SNPs) in CSL regulatory regions that are present with a different frequency among populations, taking advantage of several bioinformatic tools. SNPs are basically differences in the DNA sequence that occur in individuals and involve the mutation of one nucleotide into another. Most of these SNPs are common, and there are patterns in the statistical distribution of these SNPs in populations. Normally, the change of one SNP does not affect the correct behavior of cells, but it can induce slight changes that make each individual unique. We have

identified several SNPs that are more often present in one population than another, comparing, in a first step, Caucasian and African individuals. Since all of this work was done on publicly available databases, we confirmed the predicted frequencies by sequencing the CSL regulatory regions of individuals of both African and Caucasian origin.

In a next step, we looked for the predicted transcription factor binding sites that were affected by these SNPs *in silico*. We found that some of these TFs, predicted to bind in the CSL regulatory regions and affected by the SNPs, were likely responders to cellular stress. This means that these TFs are likely the link between UVA exposition (which, as we demonstrated during the second year, is able to regulate CSL expression) and CSL downregulation.

Nothing is known about CSL transcriptional regulation, so we began investigating whether these transcription factors are indeed able to regulate CSL expression. We started with one of them and were able to show that its overexpression can downregulate CSL at the RNA level and that its silencing can induce CSL transcription.

ChIP (Chromatin ImmunoPrecipitation) experiments showed that this transcription factor can indeed bind to CSL regulatory regions in order to regulate its transcription. Moreover, other effectors of this transcription factor are able to regulate CSL when they are upregulated.

In conclusion, we have identified the pathway responsible for CSL down-regulation in response to stress, linking its downregulation to the occurrence of SCC in response to stress, due to the transformation of human dermal fibroblasts into CAFs.

GRANT “CANCER AND IMMUNOLOGY”

The role of the Notch receptor in CD4 TH17 cell 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).

Introduction

We are investigating the role of Notch1 (N1) and Notch2 (N2) receptor signaling in TH17 cell differentiation and in the development of a TH17 response. The function of TH17 cells and IL-17 in cancer appears to be context-dependent, and was reported to either promote or reduce tumor growth. The role of Notch receptor signaling in TH17 cell differentiation was investigated here *in vitro* and *in vivo*, using the murine experimental model of B16 melanoma cells and other *in vivo* models that promote a TH17 cell response. In the B16 *in vivo* model, the IL-17 secreted by TH17 cells has been reported to influence tumor development. To better define how Notch receptor signaling impacts TH17 cell differentiation, we used mice with T cell ablation of N1 and N2 (N1N2^{ΔCD4Cre}) or of the Notch transcriptional repressor (RBP-Jk).

Results after the fourth year

The role of Notch receptor signaling in TH17 cell differentiation was first investigated *in vitro*. N1 was predominantly expressed over N2 following T_H17 cell differentiation. We demonstrated that N1 and N2 receptors play an important role in the regulation of signature T_H17 cell mRNAs and IL-17A cytokine release *in vitro*, a process that may be overcome with higher concentrations of TCR-activating signaling during T_H17 cell differentiation.

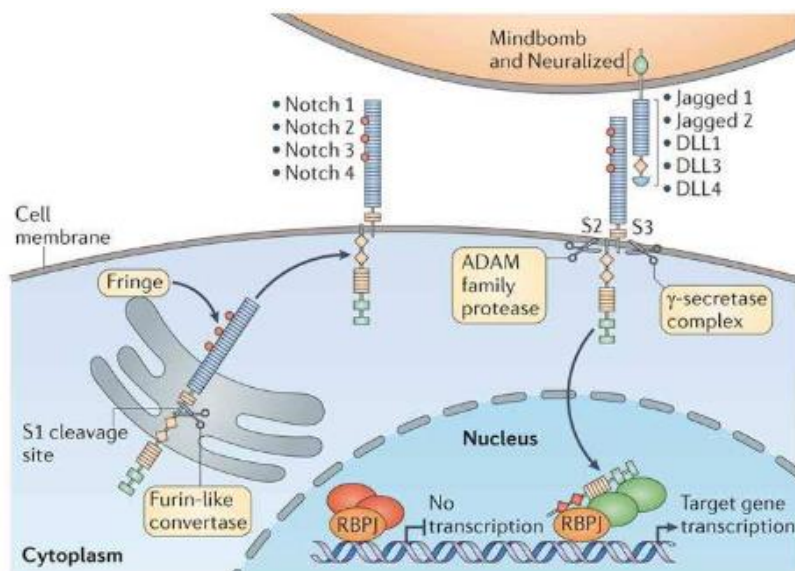


Figure 1:

From Radtke F, MacDonald HR, Tacchini-Cottier F, NRI, 2013

Notch signaling is initiated by the ligand engagement of the Notch receptor. In mammals, there are four Notch receptors (N1-4) and five Notch ligands (delta-like (Dll) 1, 3, and 4; Jagged 1 and 2). In the canonical form, the intracellular domain of Notch goes into the nucleus and binds to the transcriptional repressor RBP-Jk which displaces the co-repressor complex and activates the expression of the Notch target genes.

In contrast to the higher intracellular IL-17A levels observed in $N1N2^{\Delta CD4Cre}$ $CD4^+$ T cells following OVA/CFA immunization, reduced IL-17A and T_H17 -related cytokines were observed in antigen-specific $N1N2^{\Delta CD4Cre}$ compared to control $CD4^+$ T cells upon restimulation (Fig. 2A). N1 and N2 receptor signaling selectively promotes T_H17 -related cytokines, an effect that was confirmed using pharmacological Notch inhibitors with WT $CD4^+$ T cells (Fig. 2B). To further investigate the mechanism behind the control of cytokine secretion by Notch, we first excluded that reduced T_H17 -related cytokine levels were associated with a decrease in the proliferation or viability of restimulated Notch-deficient $CD4^+$ T cells. Furthermore, we observed no impact of *ex vivo* Notch on the mRNA expression of proteins involved in cytokine trafficking in restimulated Notch-deficient $CD4^+$ T cells. Additionally, no aberrant IL-17A subcellular localization was detected in $N1N2^{\Delta CD4Cre}$ T_H17 cells following immunization with OVA/CFA.

Finally, we have determined that the role of Notch in T_H17 cell mRNA expression and the control of T_H17 cytokine release may be overcome in other microenvironments *in vivo*, clearly suggesting a context-dependent role of Notch in T_H17 cell functions.

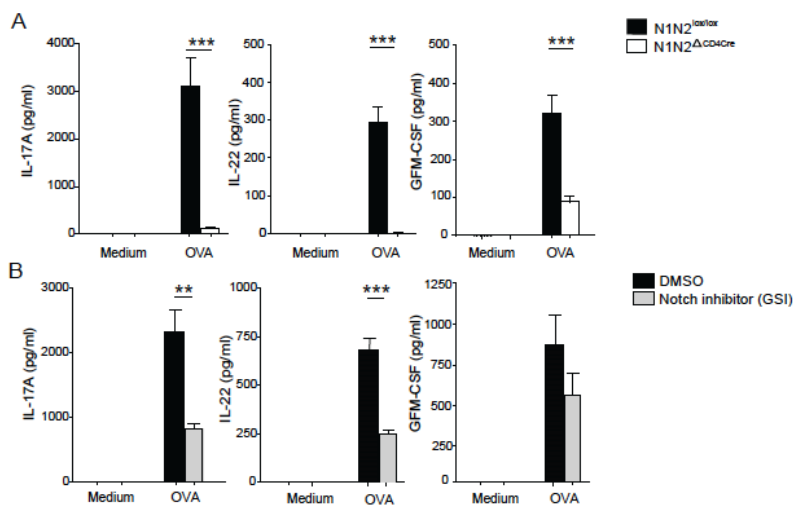


Figure 2:

9 days after OVA/CFA injection, $CD4^+$ T cells isolated from control ($N1N2^{lox/lox}$) and $N1N2^{\Delta CD4Cre}$ mice were (A) restimulated for 72 hours without ('Medium') or with OVA. (B) dLN $CD4^+$ T cells were pre-treated with DMSO or Notch inhibitor (γ -secretase inhibitor) and restimulated for 72 hours without ('Medium') or with OVA. Mean cytokine levels \pm SEM of IL-17A, IL-22 and GM-CSF analyzed by ELISA in cell-free supernatant are shown. ** $p < 0.01$, *** $p < 0.001$ versus control.

Conclusion

Collectively, our results indicate a prominent regulatory role for Notch in the fine-tuning of T_H17 cell differentiation and effector functions. Notch limits T_H17 cell differentiation at both the mRNA transcription and protein levels. In contrast, Notch controls the cytokine release by T_H17 cells, suggesting a distinct dual role for Notch in the regulation of T_H17 cells, which may be modulated depending on the environmental context. Inhibition of N1 and N2 receptor signaling might be considered in situations such as autoimmune diseases or some types of cancer in which high frequencies of T_H17 cells have a negative influence.

GRANT “CANCER AND IMMUNOLOGY”

Molecular mechanisms regulating TCR affinity-improved T cells for cancer

This “ISREC grant” amounting to CHF 80'000.- per year was awarded to [Efe Erdes](#) in July 2015 for four years.

Efe Erdes is working in the group of Prof. Nathalie Rufer (Department of Oncology, UNIL/CHUV).

1. Specific Aims

Cancer patients may have many tumor-specific T cell receptors (TCRs). Yet the avidities of these TCRs remain relatively low, eventually too low for mediating effective anti-tumor immunity. Given these limitations, we need to find better ways to determine the avidity of individual T cells, and preferentially activate T cells with optimal functional capacities. Over the past years, we have developed a unique model based on a panel of CD8 T cells expressing TCRs of increasing affinities for the NY-ESO-1 tumor antigen. It allows us to investigate the causal link between T cell avidity and T cell activation and function, and the underlying signaling mechanisms. Our work provides new evidence that the anti-tumor T cell response depends on a given TCR/pMHC affinity threshold. Our studies have revealed novel regulatory pathways and suggest that tumor-specific T cells of high avidity may not always be functionally better.

The overall goal of this grant application is to understand how T cells sense the differences in strength of TCR-peptide/MHC interactions, especially through the modulation of TCR-mediated signaling. Once identified, we will characterize the impact of blocking these molecular targets on T cell responsiveness against tumor cells, by means of *in vitro* experiments and *in vivo* in mouse models. Preliminary confidential data indicate that the Cbl proteins (c-Cbl and Cbl-b) represent important regulatory molecules in tumor-specific CD8 T cells engineered to express affinity-improved TCRs. These studies directly support the development of therapies optimizing the TCR-mediated signaling pathways, with the aim to promote therapeutic immune interventions such as adoptive T cell transfer or vaccination.

Aim 1. To dissect the impact of affinity-improved TCRs against tumor antigens on the expression of molecules involved in immune modulation/regulation (e.g. c-Cbl, Cbl-b)

Aim 2. To assess the impact of affinity-improved TCRs in blockade experiments against specific target molecules of the TCR-mediated signaling pathways (e.g. c-Cbl, Cbl-b)

Aim 3. To validate the impact of specific target proteins on affinity-improved TCRs against tumor antigens in an immunodeficient NSG mouse model

2. Background and Significance

Cytotoxic T cells recognize antigenic peptides presented by the MHC (pMHC) on the surface of infected or malignant cells via their TCR. The TCR affinity/avidity for pMHC is a key parameter for cell-mediated immunity, since strong binding to pMHC confers superior effector functions than weak interactions. This is of particular interest for immunotherapy by adoptive T cell transfer, aiming to convey immune reactivity against tumor/self-antigens, for which endogenous T cell responses are usually too weak. Indeed, adoptive cell transfer of engineered T cells augments the functional and protective capacity of tumor-antigen reactive CD8 T cells (**Fig. 1**) (1, 2). However, critical results from recent clinical trials demonstrate that affinity-enhanced engineered TCRs can lead to severe side effects in patients due to harmful cytotoxic immune responses *in vivo* (1, 3, 4). Therefore, to ensure the safety of TCR-engineered T cells in clinical trials, TCR optimization through affinity alteration must include the evaluation of optimal T cell responsiveness and lack of on-target and off-target side effects due to self-reactivity (5).

In recent years, we have established a unique panel of human CD8 T cells engineered with TCRs of progressively increasing affinity, specific for the tumor antigen NY-ESO-1 presented by HLA-A2, and designed by structure-based rational predictions (6, 7). We have found that anti-tumor T cell function can be improved by increasing TCR/pMHC affinity within physiological limits. However, further increases paradoxically lead to drastic functional declines (8, 9) (**Fig. 2**).

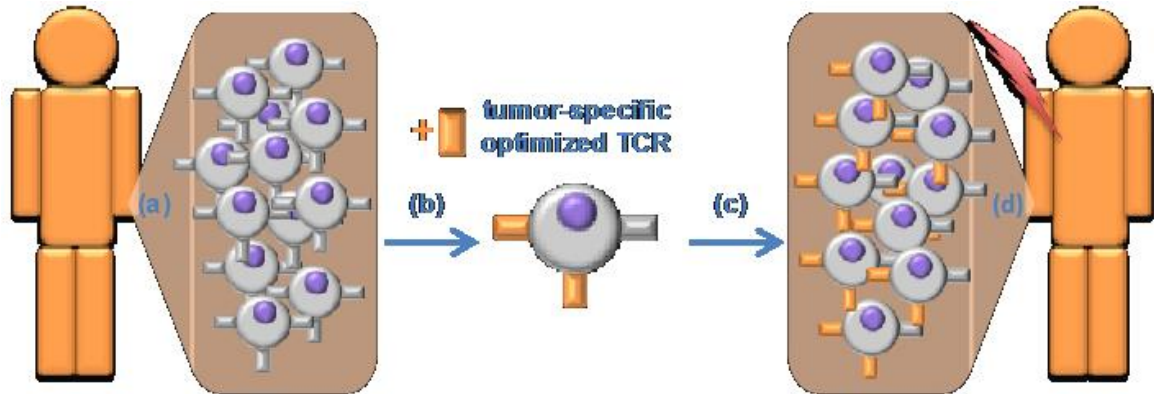


Figure 1. General outline for adoptive transfer of TCR-engineered T lymphocytes in cancer patients. PBMCs are collected (a), T cells are transduced with optimized, specific TCRs (b) followed by short-term expansion *in vitro* (c). After transient chemotherapy, expanded engineered T cells are reinfused (d).

Our study also revealed the presence of an affinity window for optimal T cell function, controlled through various molecules, such as the inhibitory receptor PD-1, and the SHP-1 and SHP-2 phosphatases (**Fig. 2**), known to regulate T cell signaling, activation and subsequent function ((10); *Presotto, Hebeisen et al., unpublished data*). Altogether, our findings suggest several levels of control in anti-cancer T cells expressing affinity-improved TCRs. Moreover, T cell activation and signaling may be limited by a given affinity threshold for TCR/pMHC interaction above which T cells may not develop productive functions.

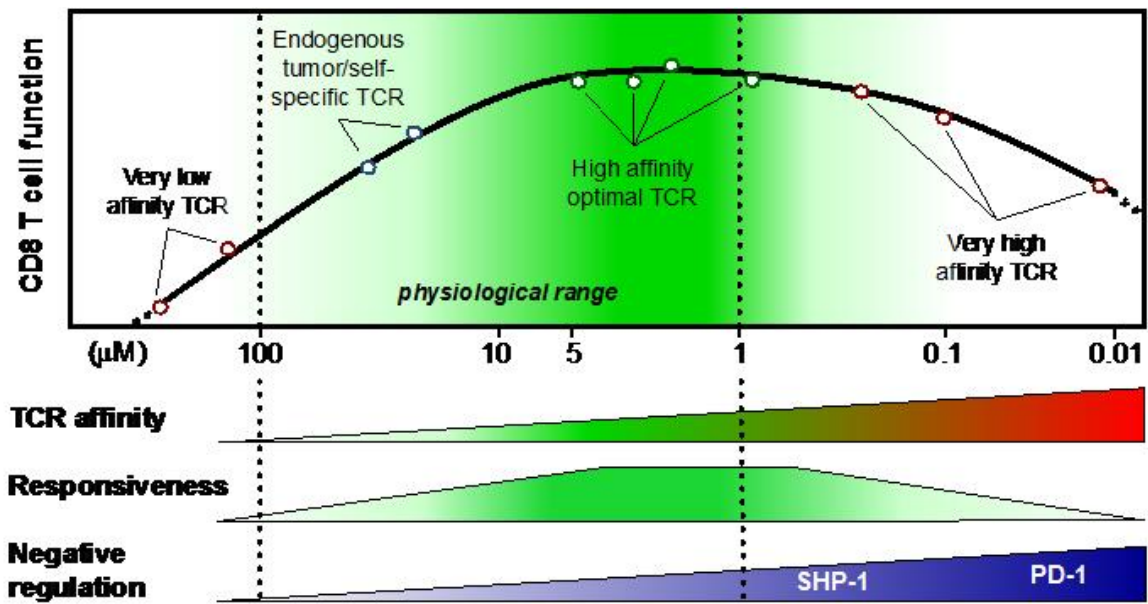


Figure 2. Model integrating the relationship between T cell function, TCR affinity and negative regulators (11). Thanks to our unique panel of CD8 T lymphocytes engineered with TCRs of progressively increasing affinity for the NY-ESO-1 tumor antigen, we were able to show that the inhibitory receptor PD-1 and SHP-1 phosphatase are involved in restricting T cell activation and responsiveness depending on TCR affinity (10, 11).

Our work further shows that TCR affinity/avidity for self/tumor-antigens needs to be carefully optimized in a step-by-step approach to (i) maximize T cell functionality, (ii) avoid toxicity associated with on-target destruction of antigen-expressing normal tissues or with loss of target specificity and (iii) minimize the up-regulation of inhibitory regulatory mechanisms (defined hereafter as immune modulation/regulation).

At present, a major objective of this grant application is to pinpoint the molecular mechanisms involved in the regulation of TCR affinity-mediated signaling and function in our panel of T cells expressing TCRs of progressively increasing affinity to the NY-ESO-1 tumor antigen. As such, we will specifically characterize the molecular players (e.g. Cbl proteins) involved in negative regulation of the TCR-mediated signaling cascade, along the TCR/pMHC affinity range, and we will modulate them in order to assess their precise impact on T cell function against tumor cells. Understanding T cell regulation and identifying tumor-specific T cells presenting optimized function directly contributes to the rational development of immunotherapy. The present study is in line with another project recently accepted by the Swiss National Science Foundation (FNS 310030-159417) that will focus (i) on dissecting the impact of TCR avidity on self-reactivity against HLA-A2, which may potentially lead to enhanced expression of immune regulators such as PD-1 and (ii) on generating new TCR variants with increased specificities towards the peptide, while avoiding HLA-A2-self reactivity.

3. Experimental Approach

The ubiquitin E3 ligases c-Cbl and Cbl-b play key physiological roles, including acting as tumor suppressors and preventing the transition from normal immune responses to autoimmune disease (12). Specifically, c-Cbl and Cbl-b act as negative regulators of TCR signaling, by targeting proteins for degradation through ubiquitination (13). C-Cbl interacts with Zap-70 and consequently promotes CD3 ζ chain ubiquitination and downregulates TCR activation during thymic positive selection. In contrast, Cbl-b is predominantly involved in down-regulating TCR signaling in mature peripheral T cells. Our current approach involves the key role of c-Cbl in the modulation of proximal TCR activation and signaling along TCR affinity. In preliminary results, we observed a progressive upregulation of c-Cbl expression in T cells with increasing affinities (*Presotto, Hebeisen, Rufer et al., unpublished data*). We hypothesize that c-Cbl acts as a TCR affinity-dependent feedback mechanism, involved in a tunable system that enables antigen-specific T cells to adapt their reactivity to different stimulatory conditions.

In the context of our first aim, we will characterize the activation states of c-Cbl and Cbl-b within CD8 T cells expressing TCR variants of increasing affinity for NY-ESO-1 at baseline and following multimer-specific stimulation (1, 5, 10 and 30 min) by flow cytometry using a phospho-flow assay recently developed in-house (14) and Western blotting (10). To do so, we will carefully evaluate the role of c-Cbl expression using a recently validated anti-phospho-c-Cbl monoclonal antibody (Tyr700; ref 558100, BD Biosciences) (*Presotto, Hebeisen, Rufer et al., unpublished data*). Similarly, we will test several mAbs against human Cbl-b either by phospho-flow assay or Western blotting, and when validated, we plan to assess the impact of affinity-improved TCRs on the expression levels of Cbl-b in our panel of TCR-transduced T cells. Moreover, we plan to characterize the expression of several molecules involved in TCR-mediated signaling, such as ZAP-70, LAT, PLC- γ , PI3K and SLP-76, that are selectively ubiquitylated by the Cbl proteins.

In the context of the second aim, we will dissect the molecular mechanisms involved in the inhibitory regulation of T cells expressing our panel of TCRs with increasing affinity for the NY-ESO-1 tumor antigen (**Fig. 2**). In collaboration with Dr. M.-A. Doucey (Dept. of Oncology, CHUV-UNIL), we will evaluate the biological significance of c-Cbl expression in these TCR affinity-improved T cells by means of transduction of a C381A c-Cbl mutant, deficient in E3 ubiquitin ligase activity or by increasing c-Cbl content using cytotolytic transduction (15). We will then characterize the functional consequence of decreasing c-Cbl enzymatic activity or increasing c-Cbl content on TCR down-modulation experiments (10), TCR-mediated signaling (e.g. pCD3 ζ , pZap70 and pERK by phospho-flow assays, *Presotto et al., unpublished data*; calcium mobilization assays (10)) and functionality (e.g. proliferation, cytokine secretion and target cell killing). We plan to perform similar experiments by over-expressing Cbl-b or blocking its expression and assessing its precise role in TCR affinity-improved T cells.

In the context of the third aim, we will characterize the *in vivo* impact of enzymatically inhibited or over-expressed c-Cbl within TCR affinity-improved CD8 T cells following adoptive transfer in immune deficient NOD/SCID/ γ c-/- (NSG) mice. Following reconstitution with these different tumor-specific T cells, the NSG mice will be engrafted with human HLA-A2/NY-ESO-1+ melanoma cell lines (Me 275 or Me 290 cells). Major objectives are *in vivo* tumor-specific T cell expansion and the study of their functional capacities for tumor recognition and eradication.

Over the past years, we have elaborated a unique and powerful model allowing us to study the precise impact of TCR/pMHC avidity on various biological parameters including cell signaling, cell activation, and function of tumor-specific T cells. A central aim is the identification of the key regulatory molecules that govern TCR affinity-mediated signaling, function and immune modulation. Identifying these molecular mechanisms (such as c-Cbl and Cbl-b as described here) highlights the intricate regulatory network that controls T cell immune responses in cancer, autoimmunity and infectious diseases. In that regard, c-Cbl represents a very interesting candidate, as this molecule is known to be recruited by inhibitory surface receptors in T cells, and was found to be up-regulated preferentially in T cells with the highest TCR affinity (*Presotto, Hebeisen et al., unpublished data*). Of particular importance is the fact that findings from such studies increase our general knowledge regarding T cell-mediated immune responses against cancer cells and help promote the development of novel immunotherapies.

References

1. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood*. 2009;114(3):535-46.
2. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, Wunderlich JR, Nahvi AV, Helman LJ, Mackall CL, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol*. 2011;29(7):917-24.
3. June CH, Maus MV, Plesa G, Johnson LA, Zhao Y, Levine BL, Grupp SA, and Porter DL. Engineered T cells for cancer therapy. *Cancer Immunol Immunother*. 2014;63(9):969-75.
4. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, Dudley ME, Feldman SA, Yang JC, Sherry RM, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother*. 2013;36(2):133-51.
5. Stone JD, and Kranz DM. Role of T cell receptor affinity in the efficacy and specificity of adoptive T cell therapies. *Front Immunol*. 2013;4:244.
6. Zoete V, Irving MB, and Michielin O. MM-GBSA binding free energy decomposition and T cell receptor engineering. *J Mol Recognit*. 2010;23(2):142-52.
7. Zoete V, Irving M, Ferber M, Cuendet MA, and Michielin O. Structure-Based, Rational Design of T Cell Receptors. *Front Immunol*. 2013;4:268.
8. Schmid DA, Irving MB, Posevitz V, Hebeisen M, Posevitz-Fejfar A, Sarria JC, Gomez-Eerland R, Thome M, Schumacher TN, Romero P, et al. Evidence for a TCR affinity threshold delimiting maximal CD8 T cell function. *J Immunol*. 2010;184(9):4936-46.
9. Irving M, Zoete V, Hebeisen M, Schmid D, Baumgartner P, Guillaume P, Romero P, Speiser D, Luescher I, Rufer N, et al. Interplay between T cell receptor binding kinetics and the level of cognate peptide presented by major histocompatibility complexes governs CD8+ T cell responsiveness. *J Biol Chem*. 2012;287(27):23068-78.
10. Hebeisen M, Baitsch L, Presotto D, Baumgaertner P, Romero P, Michielin O, Speiser DE, and Rufer N. SHP-1 phosphatase activity counteracts increased T cell receptor affinity. *J Clin Invest*. 2013;123(3):1044-56.
11. Hebeisen M, Oberle SG, Presotto D, Speiser DE, Zehn D, and Rufer N. Molecular insights for optimizing T cell receptor specificity against cancer. *Front Immunol*. 2013;4:154.
12. Mohapatra B, Ahmad G, Nadeau S, Zutshi N, An W, Scheffe S, Dong L, Feng D, Goetz B, Arya P, et al. Protein tyrosine kinase regulation by ubiquitination: critical roles of Cbl-family ubiquitin ligases. *Biochim Biophys Acta*. 2013;1833(1):122-39.
13. Barrera-Vargas A, Gomez-Martin D, and Alcocer-Varela J. T cell receptor-associated protein tyrosine kinases: The dynamics of tolerance regulation by phosphorylation and its role in systemic lupus erythematosus. *Hum Immunol*. 2014.
14. Baumgaertner P, Jandus C, Rivals JP, Derre L, Lovgren T, Baitsch L, Guillaume P, Luescher IF, Berthod G, Matter M, et al. Vaccination-induced functional competence of circulating human tumor-specific CD8 T-cells. *Int J Cancer*. 2011.
15. Brembilla NC, Weber J, Rimoldi D, Pradervand S, Schutz F, Pantaleo G, Ruegg C, Quadroni M, Harshman K, and Doucey MA. c-Cbl expression levels regulate the functional responses of human central and effector memory CD4 T cells. *Blood*. 2008;112(3):652-60.

GRANT “CANCER AND IMMUNOLOGY”

Crosstalk between T lymphocytes and melanoma cells

This “allocated grant”, amounting to CHF 40'000.- per year, was awarded to [Natalie Neubert](#) in January 2015 for one year with the support of the Zwillenberg Foundation.

Natalie Neubert is working in Professor Daniel Speiser's laboratory, Clinical Tumor Biology & Immunotherapy Group, LICR@UNIL.

Introduction

In 2008, over 67'000 new cases and over 14'000 deaths from melanoma 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.

Tumor-specific cytotoxic CD8+ T lymphocytes (CTLs) are powerful anti-tumor immune cells, because they can infiltrate the tumor microenvironment and destroy tumor cells (Figure 1). However, even following immunotherapy, the anti-tumor immune response often does not lead to complete tumor eradication, and tumors frequently relapse.

How can tumor cells survive and progress even in presence of tumor-specific CTLs? We are studying the interaction between CTLs and their target melanoma cells. Specifically, we are interested in the rapid reactions of human melanoma cells to immune attack.

Results after 4 years

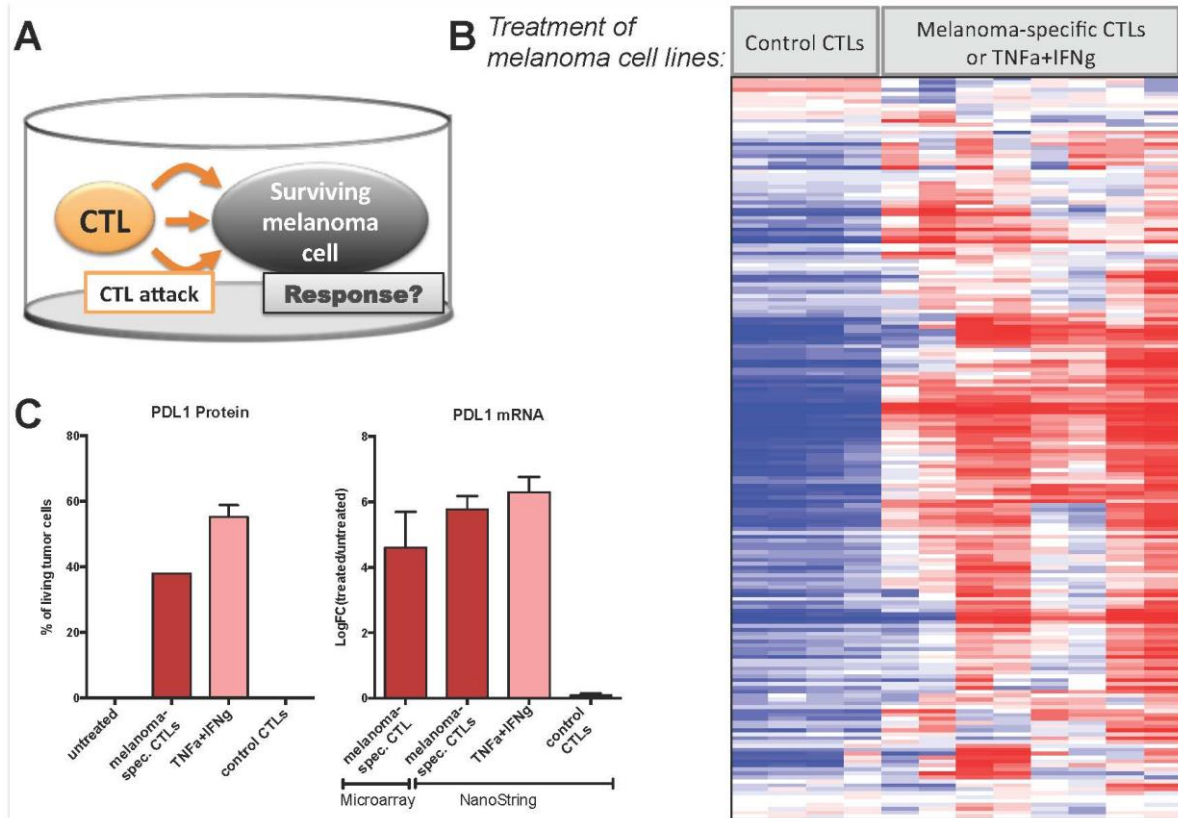
To study CTL-tumor cell interactions, we have set up a co-culture system of melanoma cell lines with melanoma-specific CTLs (Figure 2A). Melanomas develop from melanocytes, pigmented cells mostly found in the skin but also in the eye and inner ear. They express melanoma-specific antigens, such as the melanoma differentiation antigen MelanA. This antigen can be recognized by CTLs via their T cell receptor.

First, the co-cultures were screened for changes between untreated melanoma cells and melanoma cells surviving despite the presence of melanoma-specific CTLs. A genome-wide mRNA analysis showed that hundreds of genes had changed after co-culture. Three melanoma cell lines behaved similarly, suggesting that the changes are not patient-specific. The differentially expressed genes are implied in antigen presentation, interferon signaling and cell communication.

About 200 immune-related genes were chosen for further mRNA-based analyses. CTLs with irrelevant antigen specificity were used as a control. The expression of over 80 of these genes was altered in four co-cultures with melanoma-specific but none with control CTLs, demonstrating the need for an antigen-specific interaction between the CTL and the melanoma cell (Figure 2B). Interestingly, treatment of melanoma cells with TNF α and IFN γ , two cytokines typically secreted by CTLs after interaction with their target cells, could mimic melanoma response to melanoma-specific CTLs. Consequently, these factors secreted by CTLs attacking melanoma cells impact on neighboring melanoma cells, and no direct cell-cell contact is necessary to induce most of the observed changes.

In a next step, we aimed at confirming the gene expression changes at the protein level. Several tested immunomodulatory proteins were indeed increased in melanoma cells cultured with melanoma-specific CTLs but not in presence of control CTLs (Figure 2C).

To verify if our observations are restricted to the four cell lines or whether this is a general phenomenon, protein expression of sixteen melanoma cell lines was analyzed after treatment with the CTL-derived cytokines TNF α and IFN γ . While the baseline expression of some of the proteins was heterogeneous, the response to the cytokine stimulus was strikingly homogenous: only small variations between the sixteen melanoma cell lines were observed, despite their great genetic heterogeneity.



- (A) Co-culture system of melanoma-specific CTLs with melanoma cell lines.
- (B) Gene expression of 185 selected genes in melanoma cells treated with control CTLs, melanoma-specific CTLs or TNF α and IFN γ . Each column represents one melanoma cell line with the indicated treatment. Each line indicates the expression changes of one gene (in comparison with untreated melanoma cells). Color code: Red indicates genes that are upregulated in treated melanoma cells compared to untreated melanoma cells. White shows slightly upregulated genes. Blue indicates genes that are unchanged or downregulated. Data were generated using the NanoString technology.
- (C) PDL1 expression of a representative melanoma cell line cultured with the indicated treatment. Left: PDL1 protein expression measured by flow cytometry. Shown are the percent of living cells that express PDL1. Right: PDL1 mRNA expression measured by differential gene expression microarray and NanoString. Shown is the log fold increase of PDL1 expression in melanoma cells cultured with the indicated treatment compared to untreated melanoma cells.

Electron microscopy

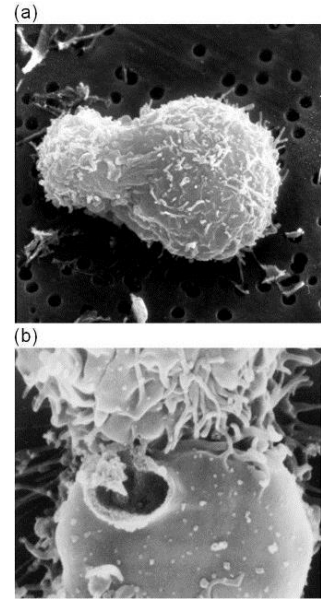
(a) Tight interaction of the T cell (left) with the large target cell (right). (b) A lethal hole in the target cell (bottom), punched by the T cell (top) already detached and on its way to other target cells (ASM MicrobeLibrary©Young).

Conclusion

Treatment with melanoma-specific CTLs, or TNF α and IFN γ , but not control CTLs induced wide changes in melanoma cells that include large numbers of biologically relevant proteins, playing multiple roles in the tumor microenvironment. The characterization of our panel of melanoma cell lines shows that our observations are a general and strikingly homogenous phenomenon, common to melanomas of different patients. Together, the identified proteins include possibly ideal targets for novel therapies against cancer.

Our findings support a dynamic interplay between CTLs and melanoma cells, driving resistance mechanisms that involve environmental factors and cells. The outcome of this project will likely contribute to our understanding of immune-related mechanisms in cancer progression and may help improve therapeutic strategies.

Two manuscripts to publish our results in scientific journals are currently in preparation.



GRANT “CANCER AND IMMUNOLOGY”

Endoplasmic reticulum stress in cancer

This “allocated grant”, amounting to CHF 40'000.- per year, was awarded to Bojan Bujsic by the EMPIRIS charitable umbrella foundation in January 2015 for one year.

Bojan Bujsic is working in the group of Prof. Fabio Martinon (Department of Biochemistry, UNIL).

Finalization of the scientific report is underway.

GRANT “MOLECULAR LIFE SCIENCES”

Spatiotemporal control of proprotein convertases at cellular and tissue levels

This “ISREC grant” amounting to CHF 80'000.- per year was awarded to [Pierpaolo Ginefra](#) in January 2013 for four years.

Pierpaolo Ginefra is working in the group of Prof. Daniel Constam (EPFL/SV/ISREC).

Introduction

Secreted enzymes of the subtilisin/kexin type proprotein convertase (PCSK) family activate or inhibit various hormones, growth factors and cell adhesion molecules by mediating endoproteolytic cleavage of their precursors after recognition of specific motifs. However, their physiological roles in most tissues and in various diseases such as cancer have remained poorly defined, in part because of technical hurdles to clearly distinguish functionally overlapping PCSK activities by conventional experimental approaches. Many of the most common and deadly human cancers (e.g. lung cancers and melanoma) produce elevated levels of usually more than one PCSK. Alterations in their abundance and in the activities of critical substrates such as TGF β or Notch generally correlate with tumor progression, invasiveness and metastatic growth. However, in order to interfere with these capabilities of cancers, a major question needs to be answered: when and where in a given tissue and in specific subcellular compartments is each of the nine PCSK family members activated and thus capable of engaging specific subsets of potential substrates? In order to develop therapeutic tools to preferentially target pathogenic PCSK functions and to reduce the toxicity of systemic PCSK inhibitors, addressing these questions will be crucial.

Previously obtained results

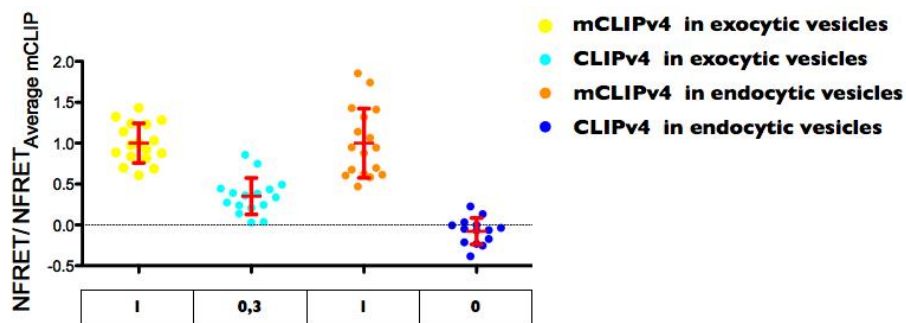
In the first year report, I showed that the PCSK FRET-based sensor CLIPv4, fused to a series of specific localization signals, was suitable to measure PC activities in different subcellular compartments in fixed HEK293T cells.

In the second year report, I showed that CLIPv4 is also suitable for live cell imaging, e.g. in the B16F1 mouse melanoma cell line.

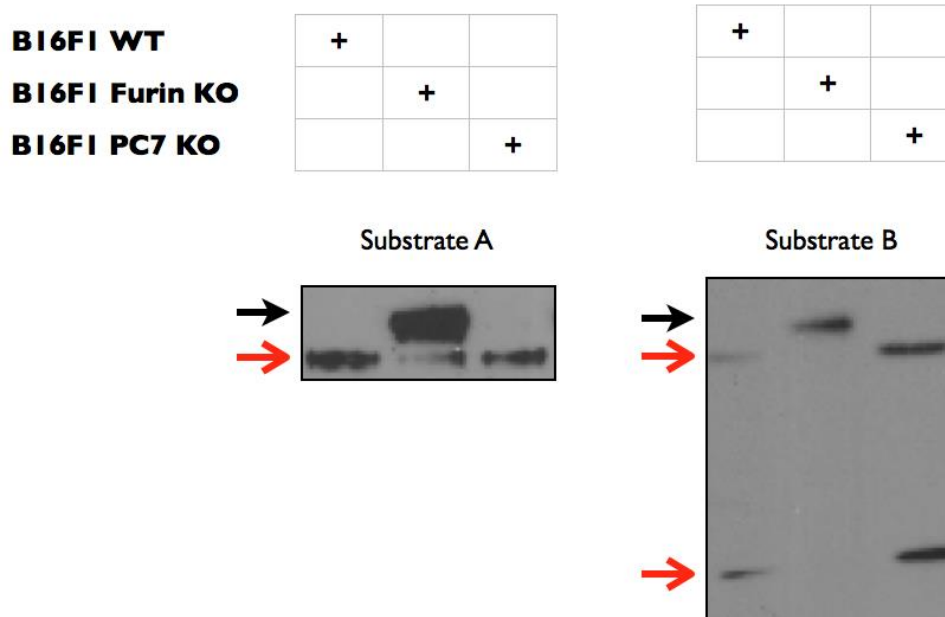
Results obtained in the third year

To determine which endogenous PCSKs are active in B16F1 melanoma cells, I tested the effect of PCSK depletion on CLIPv4 and on known cancer-relevant PCSK substrates *in vitro*. In parallel, I began investigating whether PCSKs influence syngeneic grafts of B16F1 melanoma *in vivo*. Besides validating CLIPv4 as a tool to study PCSK activity in live B16F1 cells, I previously found that the only PCSK that was active in these cells in late endosomes was Furin. I have now similarly analyzed alternative compartments. Interestingly, FRET analysis of CLIPv4 revealed significantly less endogenous PCSK activities in exocytic vesicles than in endosomes, while at the same time unmasking their identities. Moreover, pharmacological inhibition confirmed that CLIPv4 cleavage in both exocytic and endocytic vesicles is PCSK-mediated, even though the exocytic PCSK was considerably more sensitive to inhibition than the endosomal pool. To further investigate how trafficking might affect Furin and PC7 activities, I mutated known sorting motifs in their cytosolic domains. While these mutations altered the localization of overexpressed PCSK as expected, they also simultaneously altered the compartment-specific profiles of their proteolytic activities. These results provide the first direct evidence that substrate specificity and relative activity of a given PCSK depends on its subcellular localization and trafficking. In summary, my results revealed the constituents of total PCSK activity in exocytic versus endocytic vesicles and their contribution to tumor growth in the B16F1 melanoma model. Knowing where and when individual PCSKs are active will be important for future therapeutic strategies targeting such proteases.

A)



B)



Quantification of endogenous Furin and PC7 activities in exocytic and endocytic vesicles, and processing of putative substrates in B16F1 melanoma cells.

a) Normalized FRET efficiencies (NFRET) of mCLIPv4 as a positive control for maximal FRET. NFRET of CLIPv4 in the exocytic and endocytic vesicles of B16F1 cells was measured by sensitized emission as described earlier (Xia et al., 2001).

The average normalized NFRET values of mCLIPv4 and CLIPv4 are indicated in the table.

b) Western blots of know PCSK substrates. Black arrow: uncleaved substrates. Red arrow: cleaved substrate.

References

Seidah, N. and A. Prat (2012). "The biology and therapeutic targeting of the proprotein convertases." *Nature Reviews Drug Discovery* 11(5): 367-383.

Xia, Z. P. and Y. H. Liu (2001). "Reliable and global measurement of fluorescence resonance energy transfer using fluorescence microscopes." *Biophysical Journal* 81(4): 2395-2402.

Mesnard, D. and D. B. Constam (2010). "Imaging proprotein convertase activities and their regulation in the implanting mouse blastocyst." *The Journal of Cell Biology* 191(1): 129-139.

Lalou, C., et al. (2010). "Inhibition of the Proprotein Convertases Represses the Invasiveness of Human Primary Melanoma Cells with Altered p53, CDKN2A and N-Ras Genes." *PLoS One* 5(4): e9992.

GRANT “MOLECULAR LIFE SCIENCES”

The role of epithelial-to-mesenchymal transition in non-small cell lung cancer

This “ISREC grant” amounting to CHF 80'000.- per year was awarded to [Svenja Groeneveld](#) in August 2013 for four years.

Svenja Groeneveld is working in the group of Prof. Etienne Meylan (EPFL/SV/ISREC).

Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Most NSCLC patients are diagnosed at a time when they already have metastases. The epithelial-to-mesenchymal transition (EMT) is a process that contributes to metastasis formation. It usually takes place during embryo development, but can be reactivated in cancer. Increased levels of the EMT-inducing transcription factor (EMT-TF) Snail in NSCLC frequently go hand in hand with a poor patient prognosis. However, it remains largely unknown how Snail impacts the course of disease. Therefore, one aim of this project is to better understand the role of Snail in NSCLC. In addition, I am exploring the connection between the hexosamine biosynthesis pathway (HBP) and EMT. This metabolic pathway generates a product that the cell can use to modify proteins in order to transmit signals. This seems to be important during EMT.

Results

The study of Snail in mice

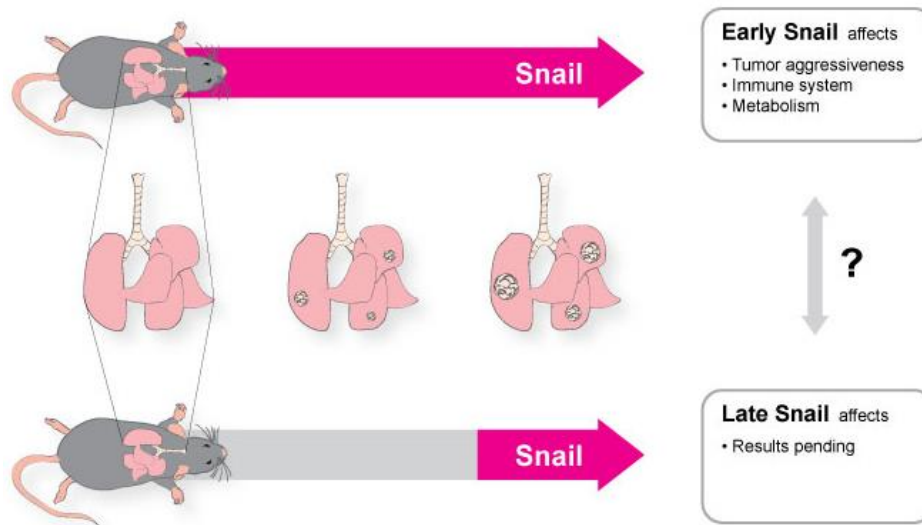
Using a sophisticated system that enables the production of a protein in a timely controlled manner, I have generated mice that have an increased amount of the EMT-TF Snail in their lung tumors. Two different schedules of Snail induction were applied. First, Snail was induced as the tumors were developing, representing its role in the early phases of the life of a tumor. Second, Snail was induced when the tumors were already established, resembling its involvement later in the tumors' life.

Snail in tumor development

Early Snail induction resulted in the development of fewer, but larger tumors. However, the speed of the tumors' growth was not affected. Overall, the tumors appeared more aggressive and more advanced. Accordingly, EMT and invasion into other structures, such as blood vessels and bronchi, were more frequent after Snail induction. Furthermore, the composition of the immune cells within the tumors changed. An effect of Snail on the immune system was also observed using an unbiased approach, which involved analyzing all the genes displaying altered expression following Snail induction. Many genes involved in the immune response were more active. The results of the late Snail induction are still awaiting complete analysis.

The hexosamine biosynthesis pathway

I have found that in human NSCLC cells the activation of EMT leads to an increased production of glutamine-fructose-6-phosphate-aminotransferase 2 (GFPT2), an important enzyme in the HBP. Additional experiments revealed that the level of the protein modification, for which the HBP provides the substrate, was elevated as well. Interestingly, the gain in GFPT2 production and protein modification was also observed in mouse lung tumors after early induction of the EMT-TF Snail. Conversely, a forced boost in GFPT2 production did not cause an EMT by itself. Suppressing the GFPT2 increase during EMT did not hinder the process. However, reducing GFPT2 in already mesenchymal cells rendered the cells more epithelial.



The effects of early and late Snail induction in a mouse model of NSCLC

To study the role of Snail in NSCLC, two different induction schemes were used for comparison. Top: Early induction of Snail, resembling its role during tumor development. Bottom: Late induction of Snail, mimicking its involvement in established tumors.

Summary

During my second year, I studied the EMT-TF Snail by producing it in a mouse model of human NSCLC. I found that Snail not only induced EMT, but also increased the aggressiveness of the tumors and changed the composition of the immune cell microenvironment. In addition, the metabolism of the tumors was altered. The role of the EMT-TF Snail might therefore extend beyond EMT in NSCLC. My current results regarding the HBP suggest that it supports a mesenchymal status once the transition has occurred.

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 was awarded to the research group of [Prof. Oliver Hantschel](#) (EPFL/SV/ISREC).

Introduction

Cancer is caused by defined changes in the genetic material of a cell that result in the expression of abnormal amounts of or structurally altered proteins. As a consequence, cells grow and divide infinitely and form tumors. One very important class of protein molecules with these cancer-causing properties are protein kinases. These enzymes are molecular switches that are normally in the off-position in normal cells, but always in the on-position in cancer cells. Since 2001, 30 new drugs have been discovered that are able to block the on-position of particular protein kinases and thereby confine the growth of tumors. Unfortunately, cancer patients often only benefit from these new drugs for a short time, as changes in the molecular structure of the protein kinase prevent the effective action of the drug.

The Hantschel laboratory is using novel approaches to study protein kinase signaling, with the aim of identifying innovative therapeutic approaches that may help to overcome resistance to conventional kinase inhibitors.

Results

Targeting cancer cells with tailor-made monobody protein inhibitors:

The Hantschel lab uses small engineered antibody mimics, termed monobodies, to inhibit the activity of protein kinases in different tumor types. Antibodies are widely used in oncology to target specific surface molecules on cancer cells, but cannot be used inside cancer cells due to their size and complex molecular structure. In contrast, monobodies are much smaller (less than 1/10th of the size of an antibody, see Figure 1), can be used inside cancer cells and bind their target proteins tightly and with extremely high specificity. In the past year, the Hantschel lab has identified and characterized several monobodies targeting protein kinases and related oncoproteins. Our goal is to establish monobodies as a novel class of intracellular protein-based therapeutics. We hope to kick off their use as tools beyond basic research and towards possible applications in cancer patients. This innovative endeavor addresses a central problem in cancer medicine and has the potential to provide a ground-breaking new approach to target cancer. Prof. Hantschel was awarded a Consolidator Grant from the European Research Council (ERC), providing funding from mid-2016 for 5 years that will allow to expand this line of research.

Understanding the complexity of cancer genomes:

The amount of available information on genetic alterations in cancer cells currently far exceeds our insight into the precise genetic events that ultimately lead to the development of cancer. The effective implementation of personalized medicine approaches requires that we be able to identify actionable genetic alterations within the complex genetic landscape of cancer cells. In collaboration with researchers from the Knight Cancer Institute in Portland and the Fred Hutchinson Cancer Research Center in Seattle, the Hantschel lab has recently published a study in which point mutations in the TNK2 protein kinase were identified in patients with acute myelogenous leukemia. The molecular mechanism of action was elucidated and targeting of the kinase by the drug dasatinib was established (Maxson et al. (2015) **Cancer Res.**, 76(1), 127-138).

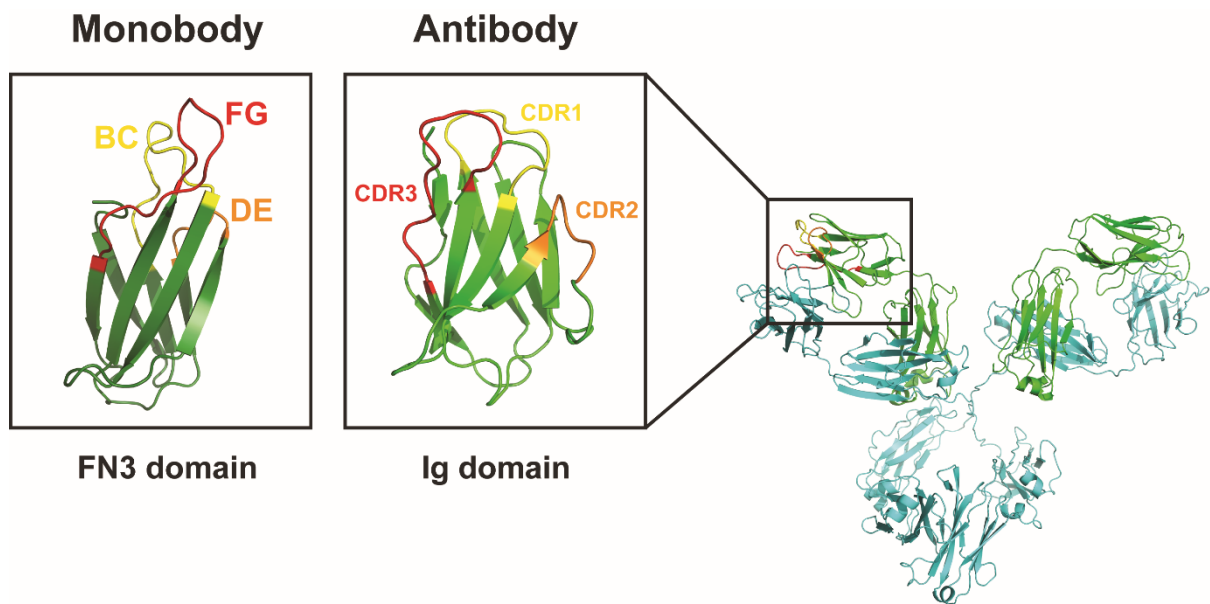


Figure legend

Comparison of the molecular structures of an antibody that is composed of immunoglobulin (Ig) domains and a monobody that is built from a fibronectin type 3 (FN3) domain.

ISREC CHAIR “FUNDAMENTAL ONCOLOGY”

Metabolic regulation and booster for harnessing anti-tumor immunity

This chair endowed with CHF 500'000.- per year for a period of six years was allocated in June 2013. It was awarded to the UNIL/LUDWIG research group of [Prof. Ping-Chih Ho](#).

Metabolic transformation is a cardinal hallmark of most cancer cells that increases their proliferative and anti-apoptotic capacity by boosting anabolic metabolism, mainly through aerobic glycolysis and glutaminolysis. Elevated aerobic glycolysis and glucose uptake ability in cancer cells fuel their unregulated proliferation by accumulating glycolytic intermediates for the generation of biosynthetic macromolecules; this property of cancer cells is well known as the “Warburg effect”. This metabolic property of cancer cells underscores the importance of glucose metabolism in cancer progression and is also the foundation of the fludeoxyglucose positron emission tomography (FDG-PET), the primary method used to identify and monitor the location and growth of primary and metastatic malignancies. *Despite this knowledge, it remains unclear whether the metabolic state(s) of cancer cells modulate(s) the functions and metabolic states of infiltrating immune and stromal cells.*

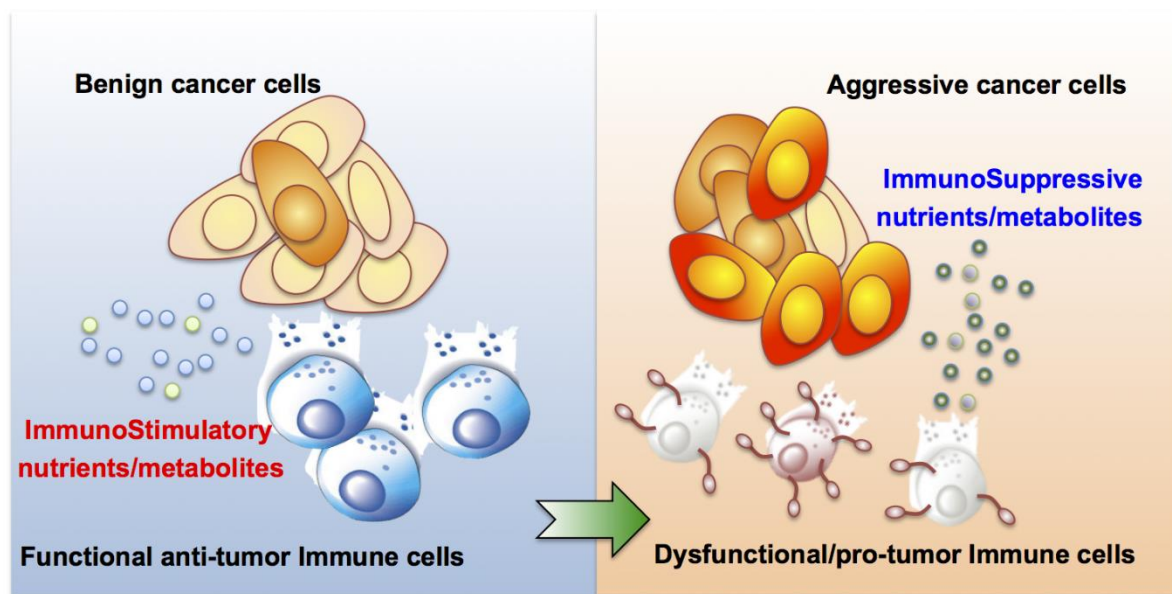
Like cancer cells, activated T cells must undergo a metabolic switch, including aerobic glycolysis and glutaminolysis, to initiate and sustain their expansion, differentiation, migration, and effector molecule production. However, a notable feature of tumor-specific T cells is that they display *increased* co-inhibitory receptor expression (e.g., PD-1, Lag3, TIM3) and *decreased* effector functions, properties commonly ascribed to “T cell exhaustion”, when these T cells infiltrate tumors. How T cells become dysfunctional and/or exhausted in the tumor microenvironment remains elusive. On the other hand, antigen-presenting cells (APCs), including dendritic cells and macrophages, also engage in aerobic glycolysis (Warburg glycolysis) to promote their activation and maturation process. Insufficient glucose or compromised glycolytic activity in these APCs leads to tolerogenic phenotypes such as reduced expression of co-stimulatory receptors and the major histocompatibility complexes I and II (MHC I and MHC II). In this proposal, we hypothesize that the tumor microenvironment may impose a nutrient-specialized/restricted microenvironment to dampen the ability of tumor-infiltrating lymphocytes (TILs) to maintain aerobic glycolysis and anabolic metabolism. This nutritional restriction may further lead to impaired anti-tumor responses of TILs and drive the accumulation of tolerogenic APCs within the tumor microenvironments. More specifically, **we aim to examine whether the high rates of aerobic glycolysis and lipid production in cancer cells initiate a “metabolic trap” for infiltrating immune cells, which facilitates immune evasion and the establishment of an immunosuppressive tumor microenvironment.** If true, this could be a general effect exploited by solid tumors to cause T cell immunosuppression when tumor-specific T cells infiltrate into tumors, and formation of tolerogenic APCs that mute T cell responses in the tumor microenvironment.

Ultimately, the goals of this proposal are to investigate this underappreciated area of tumor immunology and to apply the knowledge gained from this proposal to develop novel strategies to reawaken anti-tumor immunity with unique metabolic targeting interventions. Accordingly, the specific aims for this proposal are:

Specific Aim 1: To investigate whether elevated rates of aerobic glycolysis in tumor cells enable them to evade T cell-mediated immune surveillance.

Specific Aim 2: To develop metabolic reprogramming methods that boost anti-tumor responses in the tumor microenvironment via metabolic reprogramming of tumor-reactive T cells.

Specific Aim 3: To delineate how CD40-CD40L signaling activates tumor-infiltrating APCs by novel metabolic regulations and to determine its impacts on reawakening anti-tumor immunity.



- How does metabolic dysfunction link to immune evasion?
- How do immune cells sense and integrate metabolic activity with immune responses?
- How can we metabolically boost immune cells to improve anti-tumor responses?

The metabolic milieu of the tumor microenvironment is largely affected by the metabolic profile of tumor cells. In this proposal, we aim to determine which metabolic features must be acquired by tumor cells to lead to immune evasion. Moreover, we also aim to understand how this metabolic crosstalk between cancer and immune cells affects immune responses and the underlying mechanisms.

Publications supported by ISREC funding

Ping-Chih Ho*, Pu-Ste Liu (2016) Metabolic communication in tumors: a new layer of immunoregulation for immune evasion. *Journal for Immunotherapy of Cancer* 4; 4-12. * is corresponding author.

Wan-Chen Cheng and **Ping-Chih Ho** (2016) Metabolic tug-of-war in tumors restrains T cell anti-tumor immunity. *Onc Immunology* (In press).

Gregory Verdeil, **Ping-Chih Ho** and Daniel E. Speiser (2016) T cell function and immune regulation in cancer. *Nat. Rev. Immunol.* (Under revision).

Conference presentations supported by ISREC funding

- 2016 International Symposium of Reproduction and Metabolism, Taipei, Taiwan
- 2016 ISREC-SCCL Symposium 2016: Horizons of Cancer Biology and Therapy, Switzerland
- 2016 World Cancer Congress, Shanghai, China
- 2016 2nd Symposium Tumor Metabolism Meets Immunology, Regensburg, Germany
- 2016 5th LIMNA symposium, Lausanne, Switzerland
- 2016 Bellvitge Institute for Biomedical Research, Barcelona, Spain
- 2016 Master Class Tumor Immunology, University of Maastricht, Netherlands
- 2016 Kaohsiung Medical University, Kaohsiung City, Taiwan
- 2016 Actelion Pharmaceuticals, Basel, Switzerland
- 2016 Swiss Institute of Allergy and Asthma Research, Davos, Switzerland
- 2015 Center for Immunity and Infection, UNIL, Lausanne
- 2015 Metabolism in Cancer and Stromal Cells, Leuven, Belgium

ISREC CHAIR “TRANSLATIONAL ONCOLOGY”

Decoding the genetics of lymphoma for the development of new therapies

This chair endowed with CHF 500'000.- per year for a period of six years was allocated in November 2014. It was awarded to the research group of **Prof. Elisa Oricchio** (EPFL/SV/ISREC).

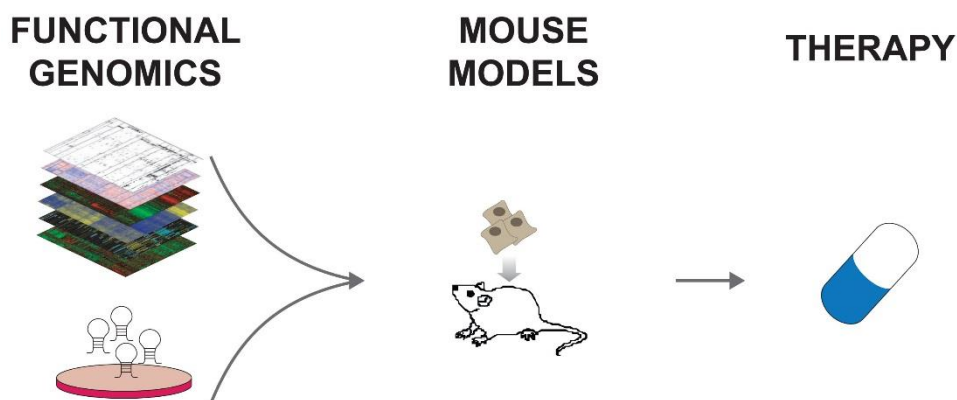
The Oricchio laboratory opened at ISREC-EPFL in November 2014. With the support of the ISREC Foundation, I have established a new research team, including two PhD students, one junior post-doc and a senior technician. The research in the lab has been mostly focused on the genetics of follicular lymphoma in order to identify novel drivers in tumorigenesis and to design new therapeutic strategies.

Introduction

Follicular lymphoma (FL) is an indolent form of non-Hodgkin lymphoma with a worldwide incidence of 120,000 new cases per year. The genetic hallmark of this disease is the chromosomal translocation t(14:18) that promotes constitutive expression of the anti-apoptotic protein Bcl2. In addition, the FL phenotype is defined by several chromosomal copy number alterations and mutations. In particular, frequently mutated genes in FL include various epigenetic regulators (e.g. MLL2 and EZH2) which modify histone methylation levels and directly and indirectly regulate the expression of several genes.

Detailed description of the project

To identify novel drivers in lymphoma pathogenesis, we have integrated genomic and genetic data of more than 300 follicular lymphoma patient samples. We have identified a novel gene called Sestrin1 as a target of recurrent copy number losses in chromosome 6q and of epigenetic silencing by mutated EZH2 in indolent and transformed follicular lymphoma. In our lab, we have chosen the vavP-Bcl2 mouse model to study the impact of specific genetic alterations on follicular lymphoma development and progression. Using this model, we have demonstrated that loss of Sestrin1 accelerates lymphomagenesis, suggesting that Sestrin1 acts as a tumor suppressor gene in follicular lymphoma. Moreover, we are testing the efficacy of a novel EZH2 inhibitor which was developed by the GlaxoSmithKline pharmaceutical company and is currently the subject of clinical trials for the treatment of lymphoma. We have demonstrated that Sestrin1 is an important mediator of therapeutic efficacy of the EZH2 inhibitor and that loss of Sestrin1 can limit its efficacy. In this study, we have identified Sestrin1 as a nodal connector between genetic and epigenetic alterations and translation regulation in lymphoma. We are completing this study and will submit the manuscript for peer-revision.



In the long-term, we intend to characterize novel genomic lesions in cancer to design novel rational therapies.

Future plans

In the next year, we aim to characterize the impact of multiple concurrent alterations on lymphoma development and progression using new CRISPR/Cas9 genome editing technology to induce overexpression of oncogenes and deplete tumor suppressors *in vitro* and *in vivo*. We will use the lymphoma vavP-Bcl2 mouse model to mimic the indolent phase of the disease and Eu-myc to study lymphoma transformation driven by myc overexpression. We intend to 1) define the impact of concurrent loss of multiple candidate tumor suppressor genes on lymphoma development and progression, 2) determine how the single and dual alterations influence downstream signaling and 3) understand how these genomic alterations affect treatment response to specific inhibitors.

FUNDS

FUND “TRANSLATIONAL RESEARCH – SARCOMA”**Immunomodulation of gastrointestinal sarcoma based on NK cell triggering**

Subtitle: GIST and NKp30-activating receptors

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 five years.

Unit INSERM U1015 and Center of Clinical Investigations IGR/Curie

Director: **Prof. Laurence Zitvogel and Alexander Eggermont**, IGR - Institute Gustave Roussy

Introduction

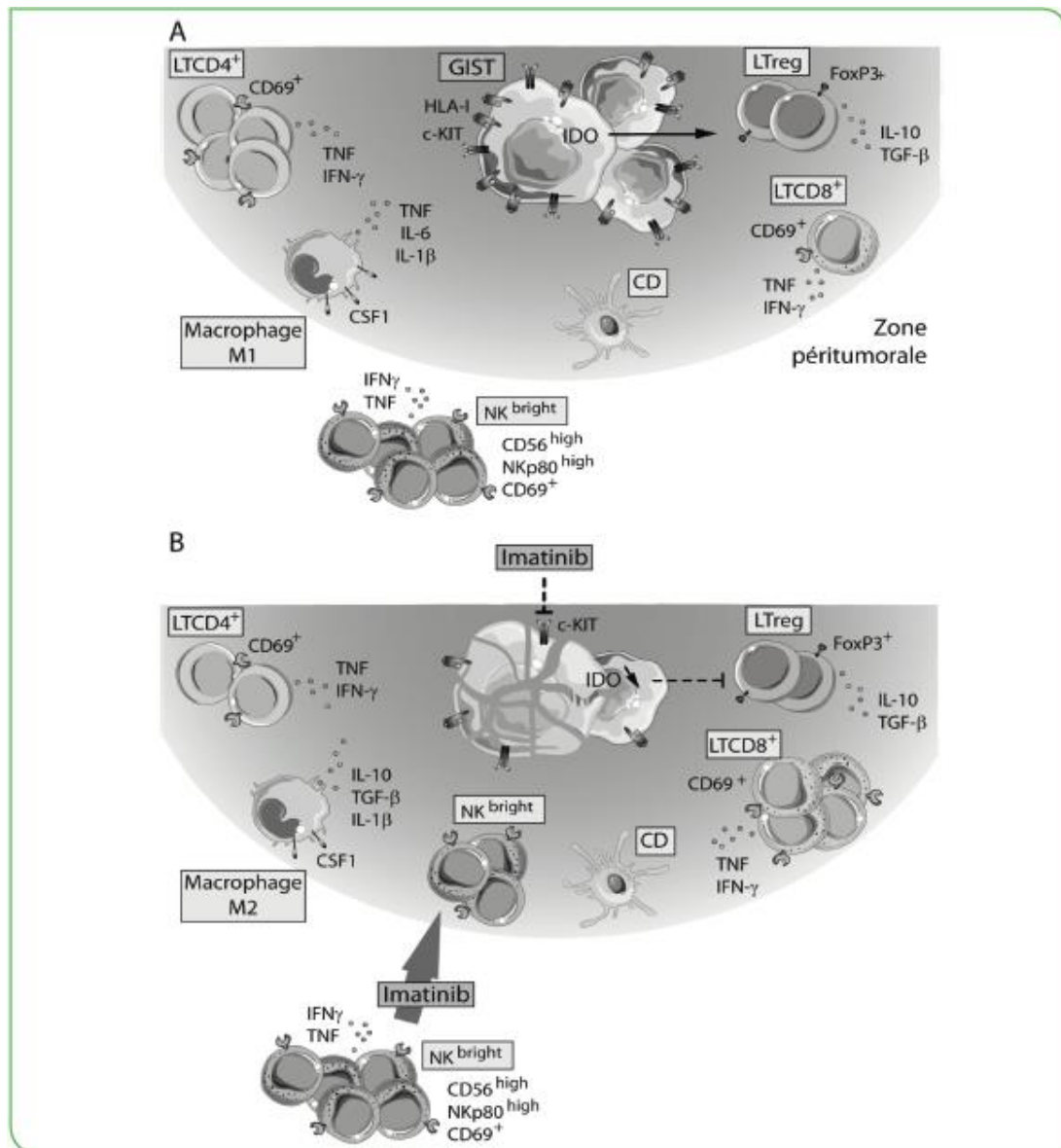
Our laboratory has demonstrated that gastrointestinal sarcomas (GIST) are controlled by the immune system, mostly by NK cells and their major activating receptor, NKp30. The paradigmatic therapy, Glivec/imatinib mesylate, an inhibitor of the aberrant oncogenic tyrosine kinase c-KIT or PDGFRA, exhibits off-target effects on the immune system, boosting dendritic cell-NK cell cross talk and leading to a strong potentiation of the direct tumoricidal activity of Glivec. Despite the breakthrough status of this approved drug, cures are hardly attained and the long-term partial remissions give rise to secondary mutations in the oncogene that are barely amenable to therapeutic control. Therefore, immuno-oncological management of GIST, preferentially based on NK-cell targeted immunomodulators, must be developed after the first year of control by imatinib mesylate. The aim of our research was to analyze how NKp30 might serve as a predictor of late relapse and to determine which immunosuppressive pathway could be harnessed for immuno-oncological purposes in this disease.

Material and methods

NKp30 profile determination was performed by means of qPCR on blood leukocytes, using specific primers to amplify and quantify each of the three NKp30 isoforms. More than 160 metastatic and 80 locally advanced GISTs were analyzed in a test and validation cohort. The ligands for NKp30 were measured by ELISA in the serum. Fresh GIST tumors were dissociated and incubated with various monoclonal antibodies to analyze the reactivity of tumor-infiltrating lymphocytes. Statistical analyses, carried out by expert biostatisticians, were performed with multivariate Cox regression analyses, taking into account the conventional prognosis parameters and the new immune factors.

Summary

Despite effective targeted therapy acting on *KIT* and *PDGFRA* tyrosine kinases, gastrointestinal stromal tumors escape treatment by acquiring mutations conveying resistance to imatinib mesylate (IM). Following the identification of NKp30-based immunosurveillance of GIST and the off-target effects of IM on NK cell functions, we have investigated the predictive value of NKp30 isoforms and NKp30 soluble ligands in blood for the clinical response to IM. The relative expression and the proportions of NKp30 isoforms markedly impacted both event-free and overall survival in two independent cohorts of metastatic GIST. Phenotypes based on a misbalanced NKp30B/NKp30C ratio (ΔBC^{low}) and low expression levels of NKp30A were identified in one third of patients with dismal prognosis across molecular subtypes. This ΔBC^{low} blood phenotype was associated with a pro-inflammatory and immunosuppressive tumor microenvironment. In addition, detectable levels of the NKp30 ligand sB7-H6 predicted a worse prognosis in metastatic GIST. Soluble BAG6, an alternate ligand for NKp30, was associated with low NKp30 transcription and had additional predictive value in GIST patients with high NKp30 expression. Such GIST microenvironments could be rescued by a therapy based on rIFN- α and anti-TRAIL mAb as well as IL-10 blockade which reinstate innate immunity. This work has been accepted for publication in *Oncoimmunology*, December 2015, and a review article in *Nature Review Clin Oncol* is in press.



Natural and IM-induced immunosurveillance in GIST

A. Natural immunosurveillance in GIST. GISTs contain Treg, as well as CD4⁺Th1 and CD8⁺Tc1 cells located in the tumor nests surrounded by CD56^{bright} CD16^{dim}-NK cells.

B. Effects of IM in the tumor microenvironment. IM promotes a loss of MHC class I molecules (which may reflect a T cell-based immunoediting process), a reduction of intratumoral regulatory T cells and a relocation of NK cells from the stroma to tumor foci, leading to a marked increase of the NK/Treg ratio *in situ*. The IM-induced increase of the NK/Treg ratio appeared to be more pronounced in the subset of GIST harboring an exon 11 KIT mutation (Rusakiewicz et al., Oncoimmunology, 2015). Balachandran et al. also reported a positive relationship between the IDO enzymatic activity and Treg accumulation post-IM in resectable GIST (Delahaye N., Nat Med 2011).

Selected Publications:

Zitvogel L, Rusakiewicz S, Routy B, Ayyoub M, and Kroemer G. The on and off target effects of the first Precision Medicine: the tyrosine kinase inhibitor Imatinib mesylate. *Nat. Rev. Clin. Oncol.* In press.

Rusakiewicz S, A. Perier, M. Semeraro JM, Pitt, EP von Strandmann, KS Reiners, S. Aspelagh, C. Pipérogliou, F. Vély, A. Ivagnes, S. Jegou, N. Halama, L. Chaigneau, P. Validire, C. Christidis, T. Perniceni, B. Landi, A. Berger-N. Isambert, J. Domont, S. Bonvalot, P. Terrier, J. Adam, JM Coindre, JF Emile, V. Poirier-Colame, K. Chaba, B. Rocha, A. Caignard, A. Toubert, D. Enot, J. Koch, A. Marabelle, M. Lambert, S. Caillat-Zucman, S. Leyvraz, C. Auclair, E. Vivier, A. Eggermont, C. Borg, JY Blay, A. Le Cesne, O. Mir, L. Zitvogel. NKp30 isoforms and NKp30 ligands are predictive biomarkers of response to imatinib mesylate in metastatic GIST patients. *Oncoimmunology*, in press.

Rusakiewicz, S. et al. Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors. *Cancer Res* **73**, 3499-510 (2013).

Semeraro M, Rusakiewicz S, Minard-Colin V, Delahaye NF, Enot D, Vély F, Marabelle A, Papoular B, Piperoglou C, Ponzoni M, Perri P, Tchirkov A, Matta J, Lapierre V, Shekarian T, Valsesia-Wittmann S, Commo F, Prada N, Poirier-Colame V, Bressac B, Cotteret S, Brugieres L, Farace F, Chaput N, Kroemer G, Valteau-Couanet D, Zitvogel L. Clinical impact of the NKp30/B7-H6 axis in high-risk neuroblastoma patients. *Sci Transl Med.* 2015 Apr 15;7(283):283ra55.

Zitvogel L, Galluzzi L, Viaud S, Vétizou M, Daillère R, Merad M, Kroemer G. Cancer and the gut microbiota: an unexpected link. *Sci Transl Med.* 2015 Jan 21;7(271):271ps1.

Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. *Nat Rev Immunol.* 2015 Jul;15(7):405-14.

Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharaf S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Bérard M, Nigou J, Opolon P, Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamillard M, Zitvogel L. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science.* 2015 Nov 27;350(6264):1079-84.

Vacchelli E, Ma Y, Baracco EE, Sistigu A, Enot DP, Pietrocola F, Yang H, Adjemian S, Chaba K, Semeraro M, Signore M, De Ninno A, Lucarini V, Peschiaroli F, Businaro L, Gerardino A, Manic G, Ulas T, Günther P, Schultze JL, Kepp O, Stoll G, Lefebvre C, Mulot C, Castoldi F, Rusakiewicz S, Ladoire S, Apetoh L, Bravo-San Pedro JM, Lucattelli M, Delarasse C, Boige V, Ducreux M, Delalogue S, Borg C, André F, Schiavoni G, Vitale I, Laurent-Puig P, Mattei F, Zitvogel L*, Kroemer G*. Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. *Science.* 2015 Nov 20;350(6263):972-8.

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 five 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 sarcomas that bear unique chromosomal translocations, 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. The chromosomal translocations in this subset of sarcomas result in the formation of fusion genes that encode proteins, most of which function as aberrant transcription factors or transcriptional regulators. These fusion proteins are believed to underlie the pathogenesis of the corresponding sarcomas and provide a tool to explore the mechanisms that lead to specific sarcoma development. Elucidation of the mechanisms whereby sarcoma-specific fusion proteins transform primary cells entails assessment of the effect of each fusion protein on the epigenome and transcriptome of target cells and on reprogramming to generate a cellular hierarchy that appears to characterize numerous malignancies, including sarcomas. The outcome of the studies should be the identification of potential therapeutic targets that may lead to the development of new drugs for the management of sarcomas with a rational, mechanism-based approach.

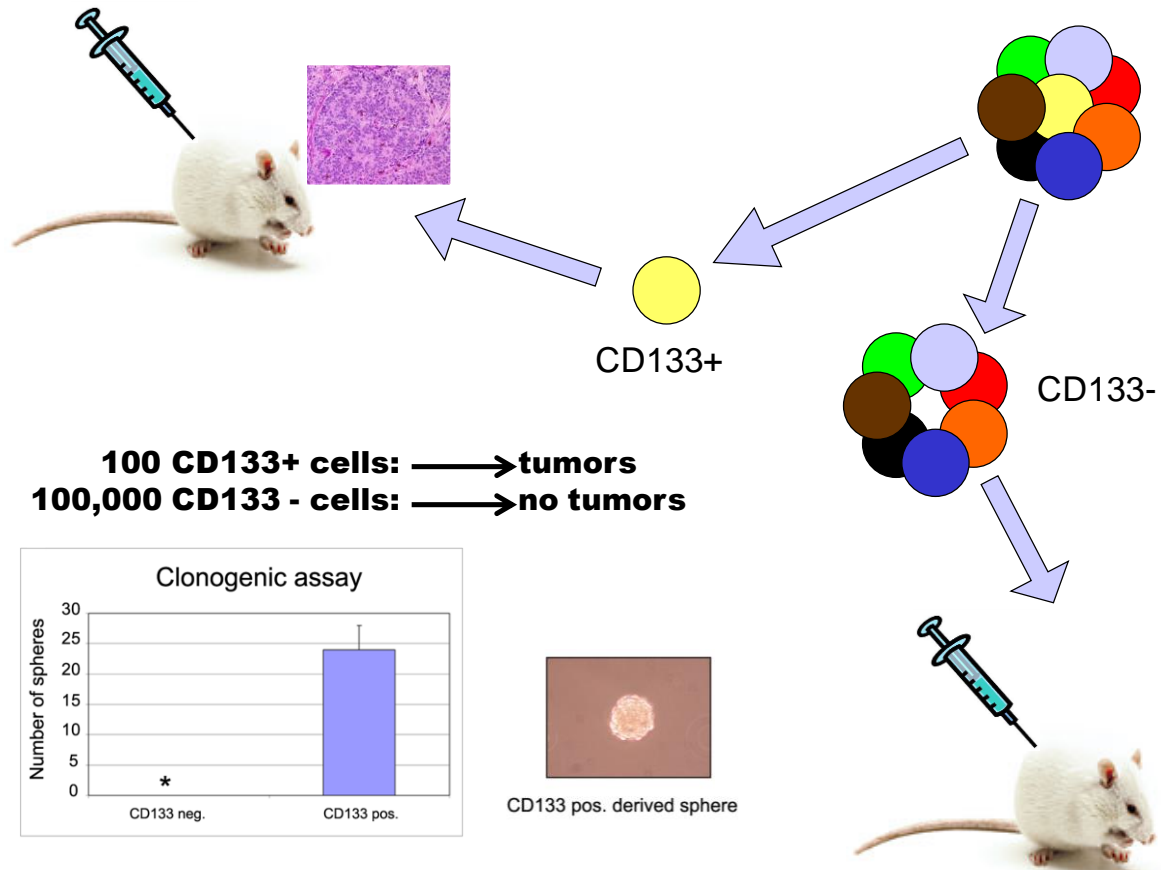
Results thus far

We have shown that bone marrow-derived mesenchymal stem cells (MSC) are the cells of origin of Ewing’s sarcoma (ES), the second most common bone malignancy of children and young adults. We have found that the fusion gene EWS-FLI1, that is characteristic of ES, induces a series of epigenetic modifications in MSCs 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. Modulation of the miRNA expression profile leads to the emergence of cancer stem cells (CSC) in ES. CSCs are believed to constitute the driving force in most malignancies since they have the ability to self-renew and to give rise to more differentiated cancer cell progeny that constitutes the tumor bulk. CSCs are relatively resistant to conventional anti-cancer therapy and are responsible for relapse following treatment.

Results during the 4th year of funding

We have launched a comprehensive assessment of epigenetic changes that drive or maintain the CSC phenotype. Using purified CSCs from several primary Ewing sarcoma samples and chromatin immunoprecipitation followed by sequencing (ChIP-seq), we have systematically begun defining the histone modifications that distinguish CSCs from the tumor bulk. This work will identify epigenetic mechanisms that lead to CSC maintenance and that, in addition to changes in miRNA maturation, specify CSC behavior.

We have also focused on the pathogenesis of synovial sarcoma (SS), a highly aggressive malignancy occurring primarily in young adults and associated with a chromosomal translocation that generates the *SYT-SSX* fusion gene. *SYT-SSX* behaves as a transcriptional regulator but its mode of action is still obscure. We have found that *SYT-SSX* selectively activates the Wnt signaling pathway, which plays a major role in determining cellular pluripotency and participates in CSC maintenance. We are now addressing the molecular mechanisms whereby *SYT-SSX* alters Wnt signaling and have discovered that *SYT-SSX* induces major changes in chromatin structure, in a way that induces novel regulatory regions for a host of genes, many of which may be involved in transformation and reprogramming.



Isolation and assessment of CSCs in Ewing Sarcoma.

Ewing sarcoma cells are purified and stained with an anti-CD133 antibody (CD133 expression is associated with CSCs in ES). About 5-10% of the cells are CD133-positive (yellow) and these cells are sorted from the CD133-negative bulk population. Injection of as few as 100 CD133+ cells initiates formation of tumors that are a phenocopy of the original human tumor (inset top), whereas as many as 100,000 CD133- cells fail to initiate tumor growth. Single CD133+ cells generate spheres (clonogenicity test) whereas CD133- cells fail to do so (lower left inset). Image of a CD133+ cell-derived sphere (lower middle inset).

Publication:

Cironi L, Petricevic T, Fernandes Vieira V, Provero P, Fusco C, Cornaz S, Fregni G, Letovanec I, Aguet M and Stamenkovic I. The fusion protein *SYT-SSX1* employs core Wnt pathway transcription factors to induce a partial Wnt signature in synovial sarcoma. 2016, *in revision*

FUND “TRANSLATIONAL RESEARCH – CANCER IMMUNOTHERAPY” Engineering T lymphocytes for long-term cancer therapy

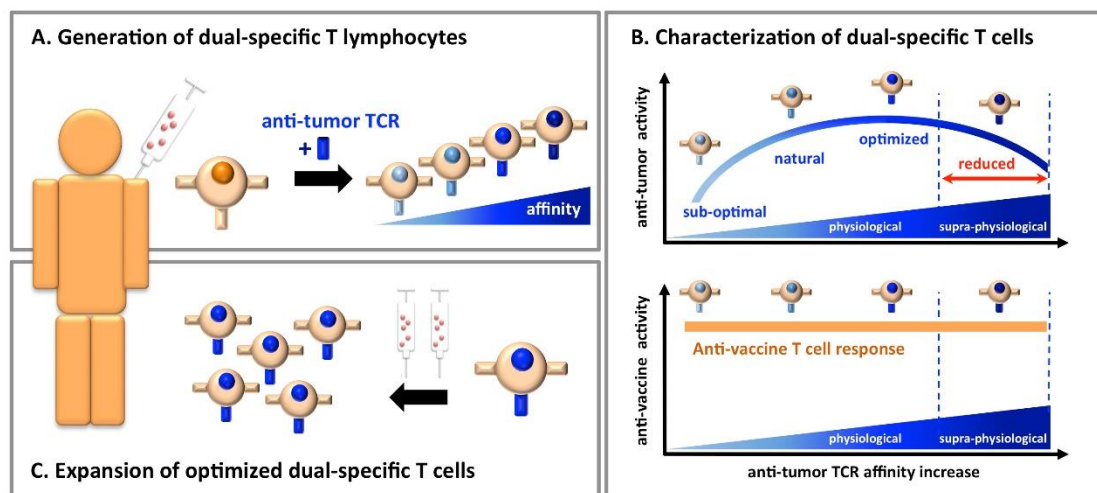
This “allocated fund” from a private donator and amounting to CHF 235'000.- was allocated in June 2013 for two years.

It was awarded to the research group of **Dr Nathalie Rufer** (LICR@UNIL).

Introduction & Objectives

Immunotherapies based on the transfer of natural tumor-specific T lymphocytes have proven to be clinically successful and safe in cancer patients refractory to conventional treatments. Unfortunately, the clinical efficacy of this approach has been hampered by the difficulty to obtain highly tumor-reactive and long-lived T lymphocytes for each patient. Alternative strategies based on the genetic transfer of tumor-specific T cell receptors (TCRs) into T lymphocytes before their re-infusion into patients have been developed. Such approaches provide the unique opportunity to strengthen cancer immunotherapy efficacy through the selection of affinity-optimized TCRs with greater potential to mediate efficient and long-lasting anti-tumor responses (Fig. 1).

This project, supported by the ISREC Foundation, aimed at exploring the feasibility and advantages of engineering vaccine virus-specific T lymphocytes to co-express affinity-optimized tumor-specific TCRs, assuming that such dual-specific (anti-tumor & anti-vaccine) T cells could benefit from the ability of vaccine-induced memory to establish more long-lasting and protective anti-tumor responses. Moreover, we wished to investigate the optimal affinity window of anti-tumor TCRs when expressed in a vaccine virus-specific cellular background.



Principle and engineering process of dual-specific T cells for cancer immunotherapy. (A) Vaccine virus-specific T lymphocytes were first isolated from a healthy donor previously immunized with the yellow fever vaccine. Next, vaccine virus-specific T cells were engineered with a panel of anti-tumoral TCRs of incremental affinity. (B) Anti-tumor TCR-engineered vaccine virus-specific T lymphocytes displayed dual-specific polyfunctionality. (B, upper graph) Tumor-specific function was limited to a given affinity threshold, above which T cells were unable to develop productive anti-tumor function. (B, lower graph) In contrast, when dual T cells were stimulated with the vaccine virus-specific antigen, all engineered T cells, including the very high affinity anti-tumoral cells retained their functional competence. (C) Dual-specific (tumor & vaccine) cells expanded to a greater extent in response to vaccine virus than tumor-antigen specific stimulation, suggesting that vaccine boosts could potentiate their expansion *in vivo*.

Results & Perspectives

Taking advantage of a well-established panel of tumor-specific TCRs of progressive affinity (1, 2), T lymphocytes induced by the yellow fever vaccine virus and collected from healthy individuals (3) were first engineered to co-express dual (anti-tumor & anti-vaccine) TCRs (Fig. 1A). We further showed that vaccine virus-specific T cells could be reprogrammed into highly polyfunctional dual-specific (anti-tumor & anti-vaccine) memory cells (Fig. 1B, upper graph). We demonstrated that enhancing tumor-specific TCR-affinity within physiological limits could optimize the anti-tumor function of such dual-specific T lymphocytes. However, in line with our previous report (1), a further anti-tumor TCR-affinity increase led to the up-regulation of several inhibitory receptors and to drastic anti-tumor function declines (Fig. 1B, upper graph). Interestingly, T cells of very high anti-tumor TCR affinity retained their functional competence when stimulated by the vaccine virus antigen (Fig. 1B, lower graph). Altogether, our data demonstrated that tumor-specific functions in dual T cells were limited to a given affinity threshold, above which T cells were unable to develop productive anti-tumor responsiveness, while not affecting the endogenous vaccine reactivity. These results further suggested that the mechanisms underlying this high-affinity-associated loss of function might be directly regulated at the TCR level rather than at the cellular level. Finally, we showed that dual-specific T cells expanded to a greater extent in response to vaccine virus than to tumor antigen stimulation, suggesting that successive vaccine boosts could potentiate their expansion and therapeutic activity *in vivo* (Fig. 1C).

In summary, our results highlight the benefits and advantages of engineering vaccine virus-specific T lymphocytes with affinity-optimized TCRs to achieve optimal and long-term anti-tumor function (4) and provide a promising rationale for the development of effective immunotherapeutic cancer strategies.

References:

- (1) Hebeisen M, Baitsch L, Presotto D, Baumgaertner P, Romero P, Michielin O, Speiser DE, and Rufer N. SHP-1 phosphatase activity counteracts increased T cell receptor affinity. **J Clin Invest.** 123(3):1044-56. 2013
- (2) Hebeisen M, Schmidt J, Guillaume P, Baumgaertner P, Speiser DE, Luescher I, and Rufer N. Identification of rare high avidity tumor reactive CD8 T cells by monomeric TCR-ligand off-rate measurements on living cells. **Cancer Res.** 75(10):1983-91. 2015
- (3) Fuertes Marraco SA, Soneson C, Cagnon L, Gannon PO, Allard M, Maillard S, Montandon N, Rufer N, Waldvogel S, Delorenzi M, and Speiser DE. Long-lasting stem cell-like memory CD8 T cells with high "naïveness" upon yellow fever vaccination. **Sci Transl Med.** 7(282):282ra48. 2015
- (4) Allard M, Fuertes Marraco SA, Gannon P, Hebeisen M, Speiser DE and Rufer N. Affinity-optimized TCR-engineering of dual tumor and vaccine virus-specific CD8 T cells for cancer immunotherapy. *Manuscript in preparation*

FUND “FUNDAMENTAL RESEARCH”

Analysis of genomic instability in normal and cancer cells studied *ex vivo*

Collaboration between the EPFL and the UNIGE

This “allocated fund” from the Fondation de Bienfaisance Pictet and amounting to CHF 100'000.- per year was allocated in September 2014 for three years.

It was awarded to the research laboratories of [Prof. Joerg Huelsken](#) (EPFL/SV/ISREC).

This project aims to answer two fundamental questions in cancer biology: Do cancers have an increased rate of acquisition of point mutations? And if so, what are the mechanisms leading to such increased rates of acquisition of point mutations? We have previously shown that human colon adenoma polyps accumulate point mutations at a higher rate in precancerous cells than in normal cells (1).

We estimated the increase in mutation rate to be about 200-fold higher and attributed this increase to DNA replication stress (1). In humans, it is difficult to obtain very early cancer samples. These are however required in order to be able to exclude that these higher mutation rates are due to mixtures of clones evolving in parallel. We therefore wanted to extend these studies to mice, where we can examine the mutation load in single clones isolated from normal tissue or cancers located in colon crypts. Specifically, 5-month-old APC-MIN heterozygous mice were used to obtain single crypts prepared from colon tissue that morphologically appeared to be cancer-free, and from colon cancers that had developed in these mice.

The mutated MIN allele which gives rise to a truncated APC protein is highly relevant for these studies since it closely resembles a mutation which is highly prevalent in colon cancer patients. The crypts were expanded in organoid tissue culture for periods ranging up to 4 months. Part of the culture was used to prepare genomic DNA at 1, 2 and 4 months. These organoid cultures make it possible to indefinitely produce primary, normal cells, since they can preserve the normal tissue organization as it is found *in vivo*, including the full differentiation program and the typical morphologies of colon epithelial cells.

As expected, we found that normal crypt organoids and cancer crypt organoids have different morphologies. While initially the normal MIN^{+/-} crypts had the morphology of normal colon epithelial cells, after 4 months most of these organoid cultures exhibited a morphology typical of cancer cells. This suggests that normal cells from the APC-MIN mice are either not entirely normal or undergo further changes *in vitro* (possibly loss of the wt allele by loss of heterozygosity).

We therefore decided to include crypts from wild-type mice in our further studies. After isolation, the genomic DNA was subjected to exome capture and sequenced in an Illumina next generation sequencer. The analysis of the samples is ongoing.

We expect to complete the sequence analysis of multiple crypts from cancer and normal tissue (at least twenty) in the next year. When completed, this study will allow us to calculate mutation rates in normal and cancer colon tissues, as we had proposed.



(1) Nikolaev SI, Sotiriou SK, Pateras IS, Santoni F, Sougioultzis S, Edgren H, Almusa H, Robyr D, Guipponi M, Saarela J, Gorgoulis VG, Antonarakis SE, Halazonetis TD. A single-nucleotide substitution mutator phenotype revealed by exome sequencing of human colon adenomas. Cancer Res 72: 6279-6289, 2012.

ORGANISATION

Founded on June 18, 1964, the ISREC Foundation is a private non-profit foundation.

Its mission is to select and support translational cancer research projects and, in doing so, to forward the transfer of knowledge and to encourage collaborations between fundamental and clinical research. The goal of these innovative projects is to translate discoveries into results and to generate a positive impact on the future treatment of human cancer.

The Foundation is composed of:

THE FOUNDATION COUNCIL

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

President

Mrs. Catherine Labouchère

Jurist / Lawyer, Delegate of the Canton of Vaud parliament

Members

Mr. Yves Henri Bonzon

Head Investment Management Julius Bär / CIO and member of the Executive Board

Prof. Franco Cavalli

Representative of the Scientific Board, Scientific Director, IOSI (Istituto Oncologico della Svizzera Italiana, Bellinzona)

Prof. Pierre-Marie Glauser

Lawyer and professor of tax law at the UNIL (University of Lausanne), Associate at Abels Oberson SA

Prof. Pierre-François Leyvraz

General Director, CHUV (Centre Hospitalier Universitaire Vaudois)

Prof. Philippe Moreillon

Vice-rector, UNIL (University of Lausanne)

Mr. Thomas Werner Paulsen

CEO, Chief Financial Officer, Head of Finance and Risk Division, Banque Cantonale Vaudoise, Lausanne

Mrs. Béatrice Schaad

Director of the Communications Department, CHUV (Centre Hospitalier Universitaire Vaudois)

Prof. Didier Trono

Full Professor, GHI (Global Health Institute), EPFL (École Polytechnique Fédérale de Lausanne)

Prof. Thomas Zeltner

Former Director Federal Office for Public Health

THE SCIENTIFIC BOARD

The Scientific Board is composed of experts of international renown in various fields of cancer research.

President

Prof. Franco Cavalli

Scientific Director, IOSI (Istituto Oncologico della Svizzera Italiana)

Members

Prof. Adriano Aguzzi

Director, Institute of Neuropathology, University Hospital of Zurich

Prof. Martin Fey

Director, Clinic and Policlinic for Medical Oncology, Inselspital - Bern University Hospital

MANAGEMENT

Assisted by the Scientific Board, the Management selects the research projects to be supported, and presents its proposals to the Foundation Council. It develops and proposes a strategy of fundraising and assumes the tasks allotted by the regulations of the Foundation.

Prof. Francis-Luc Perret

Director

THE FINANCIAL AUDITORS

The financial auditors, whose tasks are determined by law, are nominated by the Foundation Council. They are elected for one year. The 2015 mandate was entrusted to **EY**, 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 31 2015, the fortune of the foundation amounted to CHF 64 million.

Total subsidies remitted in 2015	CHF	2'456'000
in support of scientific and academic training	CHF	353'000
Grant "Cancer and immunology"		balance paid in 2014
Grant "Cancer and immunology"		balance paid in 2014
Grant "Cancer and immunology"	CHF	80'000
Grant "Cancer and immunology"	CHF	40'000
Grant "Cancer and immunology"	CHF	40'000
Grant "Molecular Life Sciences"	CHF	80'000
Grant "Molecular Life Sciences"	CHF	80'000
12 Grants "International Summer Research Program"	CHF	33'000
in support of translational cancer research	CHF	2'103'000
ISREC Chair "Translational oncology"	CHF	500'000
ISREC Chair "Translational oncology"	CHF	500'000
ISREC Chair "Translational oncology"	CHF	500'000
Fund "Translational research – sarcoma" - IGR	CHF	200'000
Fund "Translational research – sarcoma" - CHUV	CHF	303'000
Fund "Fundamental research"	CHF	100'000
Total gifts, donations, legacies, external grants received in 2015	CHF	15'002'217
74 spontaneous gifts from private individuals	CHF	249'591
21 gifts from companies, associations, foundations	CHF	356'446
9 gifts for allocated grants / funds	CHF	7'323'672
<i>3 specific gifts for the AGORA – Cancer Center</i>	CHF	4'500'000
<i>6 gifts for grants</i>	CHF	2'823'672
48 gifts in memory of deceased people	CHF	15'524
49 legacies, successions	CHF	7'056'984
Capital of the Foundation	CHF	45'074'286
Reserved capital (Limited allocation funds)	CHF	8'914'901
Grants	CHF	480'000
Funds	CHF	1'434'901
ISREC chairs	CHF	7'000'000
Reserved capital AGORA – Cancer Center	CHF	10'231'967

SUPPORT THE ISREC FOUNDATION

MAKE A DONATION

ISREC Foundation projects are mostly financed by donations and legacies from people who are sympathetic to our cause.

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

Fondation ISREC Rue du Bugnon 21, 1011 Lausanne

CCP 10-3224-9 IBAN CH55 0900 0000 1000 3224 9

UBS, 1002 Lausanne IBAN CH11 0024 3243 G020 3554 0

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 a minimum of CHF 100.-.

Taxes at the cantonal level

The information available on the Zewo foundation web pages (www.zewo.ch) applies.

ISREC FOUNDATION TAXATION

The ISREC Foundation is recognized as a non-profit institution of public utility and is therefore exonerated from communal, cantonal and federal taxes.

BOOK OF DONORS

Since 1964, many donors have supported our cause through their gifts, subsidies or legacies and have thus contributed to the progress of cancer research.

We are very grateful and thank each one of them most warmly.

Among these donors, more than five hundred appear in our golden book:

CONTRIBUTIONS OF MORE THAN ONE MILLION FRANCS

One anonymous gift / One anonymous legacy Lausanne / Mrs. Annette B., Vevey / Mrs. Anne-Laurence B., Prévèrenge / Mrs. Hilda D., Colombier / Mr. Dimitri D., Pully / Mrs. Johanne G., Lausanne / Mrs. Jeanne H., Neuchâtel / Helmut Horten Foundation, Lugano / Mrs. Henriette H.-C., Lausanne / Mr. Jean-Pierre H., St Imier / Lartek Limited, Bermuda / Leenaards Foundation, Lausanne / Swiss Cancer League, Bern / Loterie Romande, Lausanne / Mrs. Marie M., Marin / Porthos Foundation, Vaduz / Mrs. Judith P., Lausanne / Mrs. Martine Monique R., Geneva / Mr. Eric S., Neuchâtel / Sevastopoulo Fund, Lausanne / Mr. Marc V., Lausanne / Canton Vaud

CONTRIBUTIONS BETWEEN CHF 100'000.- AND 1 MILLION FRANCS

Thirty-three anonymous gifts / Canton Aargau / Mrs. Charlotte B., Romanel / Mrs. Dina Henriette B., Vevey / Canton Bern / Mrs. Adelheid Gertrud B., Hilterfingen / Mrs. Elise B., Chailly / Montreux / Mrs. Anne B., Prévèrenge / Câbleries et Tréfileries de Cossonay / Mrs. Jeannette C., Vevey / Mrs. Anne-Marie C., La Tour-de-Peilz / Mrs. Florence Helen C., La Tour-de-Peilz / Ciba-Geigy AG, Basel / Copley May Foundation, Geneva / Mrs. Suzanne C., Prilly / Mrs. Ida d'A., Lausanne / Mrs. Simone D., Lausanne / Mr. Irmgard D., Locarno / Mr. Henri D., Monaco / Mrs. Clara D., Montreux / Mrs. Doris Ursula D., St-Sulpice / Mrs. Catherine D., Montreux / Mr. Marcel D., Lausanne / M. Damien D., Lausanne / Echec au cancer de la Broye, Payerne / Mrs. Elisabeth E., Geneva / Mrs. Bertha F., Yverdon / Alfred Fischer Foundation, Lausanne / Mrs. Lilia F., Lausanne / Canton Fribourg and Ligue fribourgeoise contre le cancer / Mrs. Esmeralda G. Lausanne / Canton Geneva / Mr. Louis G., Prilly / Mrs. Andrée Lucienne G., Pully / Gygi-Beguvin Fund, Lausanne / Mr. René H., Lausanne / Mrs. Elvine H., Montreux / Gohner Foundation, Zug / Heskem Foundation, Vaduz / Mr. Georg Philip H., Leipzig / Hoffman-La Roche & Co, Basel / Mrs. Marguerite J.-K., Lausanne / Mrs. Alice J., Pully / Canton Jura / Mrs. Consuela K., Lausanne / Lardeco Foundation, Vaduz / Municipalité de Lausanne / Mrs. Marthe L., Lausanne / Ligue vaudoise contre le cancer, Lausanne / Mrs. Yvette L., Vevey / Mrs. Laura L., Spanien / Mr. Pierre Louis L., Lausanne / Mr. Karl Heinz M., Krienz / Mrs. Marie-Louise M., Corsier / Medic Foundation, Geneva / Mrs. Odette M., Lausanne / Mr. Roland M., Cugy / Mrs. Liliane M., Lausanne / Mrs. Louisa M., Lausanne / Mrs. Marthe M., Lausanne / Mrs. Denise Alice N., Neuchâtel / Nestlé SA, Vevey / Canton Neuchâtel / Mrs. Marie-Louise P., Lausanne / Mr. Franz P., Coppet / Jacqueline Petit Foundation, Lausanne / Fondation de bienfaisance de la Banque Pictet & Cie, Carouge Ge / Mr. Pierre P., Estavayer-le-Lac / Mrs. Marthe P., Lutry / Mrs. Elisabeth P., Neyruz / Mr. Yves J. P., Verbier / Mrs. Louise Q., Renens / Mrs. Nina R., Pully / Mr. Georges R., Paris / Mr. Edouard-Marcel S., Lausanne / Mrs. Paulette S., Denens / Mr. and Mrs. S.-B., Siders / Mrs. Georgette S., Geneva / Mrs. Rosalie S., Montreux / Canton St-Gallen / Michel Tossizza Foundation, Lausanne / Miss Suzanne-Marie T., Payerne / Charles Veillon Foundation, Lausanne / Mrs. Evelyn V., Lausanne / Mrs. Nina W. Loney / Canton Wallis / Prof. Dr h.c. René W. (Castolin SA), St-Sulpice / Mrs. Gabriella Maria W., Geneva / Mrs. Henriette W., Lausanne / Mrs. Mona W., Geneva / Mrs. Gertrud Z., Münchenstein / Mr. Walther Willy Z., Montreux / Canton Zürich

CONTRIBUTIONS BETWEEN CHF 50'000.- AND CHF 100'000.-

Thirteen anonymous gifts / Mrs. Alice A., Moutier / Mrs. Yvette A., Vevey / Aiuto Foundation, Nyon / Mrs. Marie B., Pully / Mrs. Rachelle B., Montreux / Canton Basel-Land / Mr. Ernesto B., Geneva / Mrs. Liliane B., Lausanne / Mrs. Germaine B.-R., Aubonne / Mr. Giovanni B., Lausanne / Centrale Suisse des Lettres de Gages (Pfandbriefzentrale), Bern / Mrs. Violette C., Lausanne / Mrs. Alice E. C., Orbe / Mr. Marcel C., Lausanne / Mrs. Teresa C.-R., Zürich // Mrs. Femande C., Lausanne / Mrs. Martine D., Lausanne / Mr. Jean D., Biel / Mrs. Raymonde D., Morges / Mrs. Femande D.-A., Les Cullayes / Jules & Irène Ederer-Uehlinger Foundation, Bern / Emouna Foundation / Ernst & Young, Lausanne / Mrs. Marie E.-B., Crans-près-Céligny / Fabrique de Câbles Electriques, Cortaillod / Mrs. Ariette F., Vevey / Mrs. Josette F., Neuchâtel / Mrs. Dorothea G., Lausanne / Mrs. Lidia G., Echallens / Mrs. Liliane G., Aubonne / Mrs. Aline G., Kirchberg / Mrs. Claudine G., New York / Mrs. Renée H., Lausanne / Mrs. Marie Juliette Simone H., Geneva / Mr. Jean-Charles H., Geneva / Mrs. Margarete J., Lausanne / Prof. Gustave J., Zürich / Mrs. Marie-Louise J., Renens / La Suisse Assurances, Lausanne / Fondation Les Halliers Le Mont-sur-Lausanne / Mrs. Hedwige Meinrada L.-G. / Cancer league Valais, Sierre / Mrs. Raymonde M., Lausanne / Mrs. Marianne M., Lausanne / Mr. Eugen M.-M., Kilchberg / Nouvelle Cassius Foundation, Vaduz / Mrs. Andrée P., Lausanne / Mrs. Madeleine P., Bulle / Mrs. Etienne Q. da F., Lausanne / Mrs. Gabrielle R., Aubonne / Mrs. Marie R.-B.-J., Fleurier / Mrs. Anne-Marie S., Romanel / Tetra Laval International, Pully / Mrs. Anne-Marie U., La Chaux-de-Fonds / Mrs. Madeleine V., Les Paccots / Mrs. Corinne W., Lausanne / Mr. Pierre Z., Lausanne

CONTRIBUTIONS BETWEEN CHF 5'000.- AND CHF 50'000.-

Forty-eight anonymous gifts / Mrs. Marie A.-D., Lausanne / Action cancer des boulangers / Mr. Georges A., Colombier-sur-Morges / Mr. Emile A., Auvornier / Mrs Jacqueline A., Lausanne / Albion House Ltd, Lausanne / Alcoa International SA, Lausanne / Dr. Etienne A., Lausanne / André & Cie SA, Lausanne / Canton Appenzell Ausserrhodod / Association des Câbleries Suisses, Zürich / Mrs. Charlotte B., Prilly / Mrs. Yvonne Edmée B., Auvornier / Banque Vaudoise de Crédit, Lausanne / Mr. Aimé B., Boudry / Mrs. Elisabeth B., Lausanne / Mr. Maurice B., Lutry / Baumgartner Papiers SA, Lausanne / Mrs. Fidela B., Clarens / Mrs. Mireille B., Pully / Mrs. Jeanne B., Romanel / Bherma Vaduz Foundation, Neuchâtel / Mrs. Nicky B., Bulle / Mrs. Rosa B., Cossonay / Mrs. Emma B., Bern / Bobst & Fils SA, Lausanne / Mrs. Nicole B., Lausanne / Mrs. Clara B., Veytaux / Mrs. Reina B., Prilly / Boillat SA, Reconville / Mr. Ulysse B., Lutry / Mr. Bernard B., Bournens / Mrs. Odile B., Lens / Borel & Barbey, Geneva / Miss Alice and miss Hélène B., Lausanne / Brauchli SA, Lausanne / Mrs. Lucie B., La Tour-de-Peilz / Company Paul Bucher, Basel / Mrs. Dorothee B., La Chaux-de-Fonds / Mr. Louis B., Pully / J. Calley d'Epargne du District de Cossonay / Mr. Stefan C., St-Légier / Mrs. Anne-Marie C., Lausanne / Mrs. Eveline C., Ecublens / Mr. François C., Meggen / Mr. Jean C., Bern / Mrs. Nelly C.-B., Prilly / Mr. Albert B., Lausanne / Mr. Frédy C., Prilly / "Comeback" des motards, Lausanne / Copycolor SA, Renens / Miss Juliette C., Lausanne / Couvent de Sainte Ursule, Sion / Mr. Ernest C., Villeneuve / Mr. and Mrs. Ernest D., Echichens-sur-Morges / Miss Simone de M. d'A., Lausanne / Delta Securities, Gurnsey / Mrs. Yolande de M., Epalinges / Mrs. Aida de P. M., Loney / Régie De Rham, Lausanne / Mrs. Lily D., Lausanne / Mr. Xavier D., United Kingdom / Mrs Ariane D., Geneva / Mrs. Livia D., Montreux / Mr. Constant D., Lausanne / Mr. Emile D., Châtel-St-Denis / Mrs. Alice D., Lausanne / Schweizerische Stiftung für den Doron-Preis, Zug / Miss Floriane du B., Les Ponts-de- Martel / DuBois Invest LLC, Sierre / Edouard Dubied & Cie, Neuchâtel / Mr. Jean D. / Mr. Albert D., Vevey / Mr. Armand D., Penthalaz / Mr. Gian Andrea D., Epalinges / Ebauches SA, Neuchâtel / Ecole Hotelière de Lausanne / Mrs. Marie E., Vevey / Mr. Roger E., Vevey / Empiris Foundation, Zürich / Municipalité d' Epalinges / Etablissement cantonal d'assurances, Pully / Fabrique d'Assortiments Réunis, Le Locle / Fabrique de Câbles de Brugg / Mrs. Francisca F., Lausanne / Mr. Ruedi F., Gümliigen / Mr. Pierre F., Romont / Mr. Jules F., Payerne / FPH (Foundation pour le Progrès de l'homme), Lausanne / Mrs. Janine F., Yverdon / Mrs. Jacqueline F.-G., Lausanne / Galenica SA, Bern / Mrs. Genifer G., La Tour-de-Peilz / Mr. Mario G., Stäfa / Miss Germaine Marie G., La Tour-de-Peilz / Mr. Patrice G., St-Sulpice / Mr. Roger G., Loney / Canton Glaris / Golay-Buchel & Cie, Lausanne / Mrs. Violette G., Lausanne / Mr. Johannes G., Lausanne / Grande Kermesse de la jeunesse pour la lutte contre le cancer, Geneva / Mrs. Hilda G., Morges / Mr. Daniel G. / Mr. François G., Lausanne / Mr. Gérard H., Les Diablerets / Louise Helfferich Fonds, Lausanne / Mr. Gustav H.-M., Schaffhausen / Sources Minérales Henniez / Mrs. Violette H., La Tour-de-Peilz / Miss Marguerite H., Lausanne / Mrs. Yvette H., Lausanne / Mr. Ernst H., Biel / Mrs. Marylène P., Lausanne / Mrs. J. H., Geneva / Mrs. Claire-Marguerite H., Geneva / Mr. Heinz I., Lausanne / Imprimeries Réunies SA, Lausanne / Integra Biosciences AG, Wallisellen / Interfood SA, Lausanne / Mrs. Ginette I., Pully / Mr. Olivier J. G., Lausanne / Mrs. Joséphine J., Siders / Mrs. Germaine J., Renens / Mr. Hermann J., Ste-Croix / Juchum Foundation / Mrs. Elizabeth J., Montreux / Mrs. Suzanne J., Frankreich / Mrs. Betty K., Geneva / Idryma Georges Katingo Lemos Foundation, Lausanne / Mrs. Alice K., Grandvaux / Mrs. Rose K., Crans-près-Céligny / Kodak SA, Lausanne / La Bâloise Assurances, Basel / La Boutique d'Occasions, Lausanne / La Genevoise Assurances, Geneva / Mr. Charles-Edouard L., Glion / Mr. and Mrs. L.-S., Lausanne / Mr. Roger L., Lausanne / Mrs. Sandra L.T., Lausanne / Mrs. Alice L., Payerne / Leclanché SA, Yverdon / Lemo SA, Ecublens / Mr. Jean-Pierre L., Bournens / Mrs. Connie E.F. L., Zürich / Ligue genevoise contre le cancer, Geneva / Ligue tessinoise contre le cancer, Locarno / Lo-Holding Lausanne-Ouchy SA, Lausanne / Mrs. Marcelle L.-H., Montreux / Mrs. Emilie L.-M., Lausanne / Mrs. Jane L., Lausanne / Mr. Hans L.-B., Hasle b. Burgdorf / Mrs Patricia M., Basel / Mr. J.-M. M., Lausanne / Mrs. Rachel M., Vevey / Mrs. Alice M., Châteaueu d'Oex / Mrs. Francis M., Lausanne / Mrs. Marie-Claire M., Lausanne / Ernest Matthey Foundation, Pully / Mr. Pierre M., Lausanne / Mrs. Viviane M., Corseaux / Metalwerke AG, Dornach / Mr. Roland M., Grandvaux / Mrs. Marthe M.-M., Montreux / Mrs. Léonie M., Lausanne / Migros Genossenschafts-Bund, Zürich / Mr. François M., Lausanne / Mrs. Suzanne M., Renens / Mrs. Nelly M., Rossinière / Mrs. Charlotte M., Chavornay / Mrs. Angela N.-W., Bern / Mrs. Monique N., Vandoeuvres / Nutresco SA, Penthalaz / Mrs. Marie O.-C., Lausanne / Mr. Daniel O., Villars-sous-Yens / Payot SA, Lausanne / Mr. Georges P., Morges / Mr. Jean P., Lausanne / Mr. René P., Lausanne / Philipps AG, Zürich / Dr. Suzanne-Marie P.-R., Lausanne / Mrs. Ida P., Olens-sur-Lucens / Mrs. Mireille P., Pully / Mrs. Rose-Marie P., St-Aubin-Sauges / Mr. Emile P., Oron / Mr. Jules Ernest P., Orbe / The Pro Aremorica Trust / Publicitas SA, Lausanne / Mrs. Elyse P., Pully / Mrs. Jeanne P., Fribourg / Ramelet SA, Lausanne / Mrs. Angèle R., Payerne / Mr. Hansueli R., Bern / Mr. Alfred R., Aubonne / Renault Finance SA, Lausanne / Rentenanstalt, Zürich / Retraites Populaires, Lausanne / Mrs. Alice R., Lausanne / Mrs. Anne R., Lausanne / Messrs Alain & Jean-Daniel R., Bern / Mr. and Mrs. Hans & Hildegard R., Mettmensetten / Montres Rolex SA, Geneva / Rotary Club, Lausanne / Rütli Foundation, Luzern / Sagrave SA, Lausanne / Mr. and Mrs. David & Barbara S., Geneva / Sandoz SA, Basel / Mrs. Jeanne S., La Conversion-sur-Lutry / Mr. Carlo S., Montreux / Mr. G.A. S., Lausanne / Scheuchzer SA, Lausanne / Mr. Robert Charles S., Laupen / Mr. Paul-R. S., Lausanne / Mrs. Lucie S., Lausanne / Mrs. Clémence S., Lausanne / Mrs. Béatrice S., Pully / Mrs. Marguerite S., Lausanne / Mr. Olivier S., Rolle / Sicpa SA, Prilly / Siemens-Albis AG, Zürich / Skilift Parsenn-Furka Klosters AG, Davos Platz / Sobrate Foundation, Lausanne / Société de couture, Savigny / Société de Réassurances, Zürich / Société des Chaux & Ciments de la Suisse Romande, Lausanne / Société Romande d'électricité, Clarens / Soroptimist International - Union Suisse, Grandvaux / Mr. and Mrs. Joseph S.-G., Laufen / Mrs. Marie S. / Commune St-Sulpice / Mrs. Cécile S., St-Prex / Supra (SVRS), Lausanne / In memory of Mrs. Marie-Jeanne S., Mont-sur-Rolle / Mrs. Suzanne S., Lausanne / Team Girard, Puidoux / Miss Jeanne T., Lausanne / Mr. Jean T., Ste-Croix / Mr. Albert T., St-Saphorin-sur-Morges / The Rose Charitable Trust, United Kingdom / Trophée Ago, Loney / Mr. Georges T., Lausanne / Mr. Alain T., Bex / Mrs. Antoinette T., Nyon / Fondation Elisabetha et Jacques Tab, Lausanne / Mrs. Anne-Marie U., La Chaux-de-Fonds / Canton Uri / Miss Charlotte & miss Hildegard V., Davos / Mrs. Rosa V.-J., Lengnau / Mr. Benjamin V., Cully / Vaudoise Assurances, Lausanne / Mrs. Constance V., Le Mont-sur- Lausanne / Mrs. Cosette V., Givirns / Verrière de St-Prex SA / 24 Heures Presse, Lausanne / Mrs. Paulette V., Auvornier / Mrs. Nelly-Henriette V., Villeneuve / Mrs. Andrea V.D., Monthey / Wander SA, Bern / Mrs. Emmy W., St-Sulpice / Mrs. Lyana Elizabeth W., Montreux / Mr. Jacques W., Lausanne / Mrs. Geneviève W., Le Mouret / Winterthur Assurances, Zürich / WnG, Lausanne / Zellinvest SA, Geneva / Zyma SA, Nyon

ACKNOWLEDGEMENTS

WITH YOUR HELP WE CAN CARRY OUT OUR PROJECTS AND OUR MISSION BECOMES REALITY. YOUR GENEROSITY AND CONTINUED SUPPORT ARE PRECIOUS TO US.

THANK YOU VERY MUCH.

We also wish to specially thank Mrs. Aylin Niederberger, general secretary, Mrs. Virginie Porret, communication assistant, as well as our ambassadors, Mr. Didier Grobet and Mr. Jürg Karle for their faithful commitment.

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

Catherine Labouchère, President Francis-Luc Perret, Director

Publication editor **Virginie Porret**
Design Cover pages 1 and 3 **Spirale Communication visuelle**
Photography © Cover, P. 24, 26, 28, 33, 42 **EPFL SV ISREC** / P. 3 **ISREC Foundation** /
P. 5 **Trophée Ago, Team Girard** / P. 6, 7 **Behnisch Architekten** / P. 9, 10, 12, 13, 20, 21, 39 **UNIL**
P. 15, 16, 38 **UNIL/CHUV** / P. 30 **UNIL/LUDWIG** / P. 35 **IGR** / All rights reserved