

## Unraveling tumor-host interactions for new diagnostic and therapeutic opportunities

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### Cancer as a tissue disease

Cancer is a genetic disease. Genetic and epigenetic alterations activate tumor promoting genes (i.e. oncogenes, such as Ras, BCR-ABL, PI3K) and/or inactivate tumor-suppressor genes (i.e. P53, APC, PTEN) resulting in uncontrolled cell growth, escape from cell death, and immortality transforming a normal cell into a cancer cell. Most of these alterations are acquired as the result of intrinsic errors of DNA replication during adult life. They can be exacerbated by extrinsic noxes, such as UV light (i.e. sun exposure), ionizing radiations (e.g. X-ray), chemicals and materials (e.g. aniline, asbestos), or cigarette smoke, but also by intrinsic tissue events, such as persistent infections (e.g. HBV, *H. Pylori*) or chronic

inflammation (e.g. *colitis ulcerosa*). In a minority of cases, genetic mutations are inherited by germ line transmission thereby greatly increasing the risk of developing cancer early in life (e.g. BRACA1/2 for breast and ovarian cancer, APC for colorectal cancer). However, in order to generate clinically-relevant tumors and progression toward metastasis, complex heterotypic multi-cellular interactions between cancer cells and the tumor microenvironment (TME) are required. Events in the TME are essential parts of tumorigenesis and progression [1]. The TME contains many distinct cell types, including endothelial cells, pericytes, fibroblasts, polymorphonuclear leucocytes (neutrophils, eosinophils, basophils, mast cells), lymphocytes (T, B, NK cells), macrophages and dendritic cells (Fig. 1) [2].

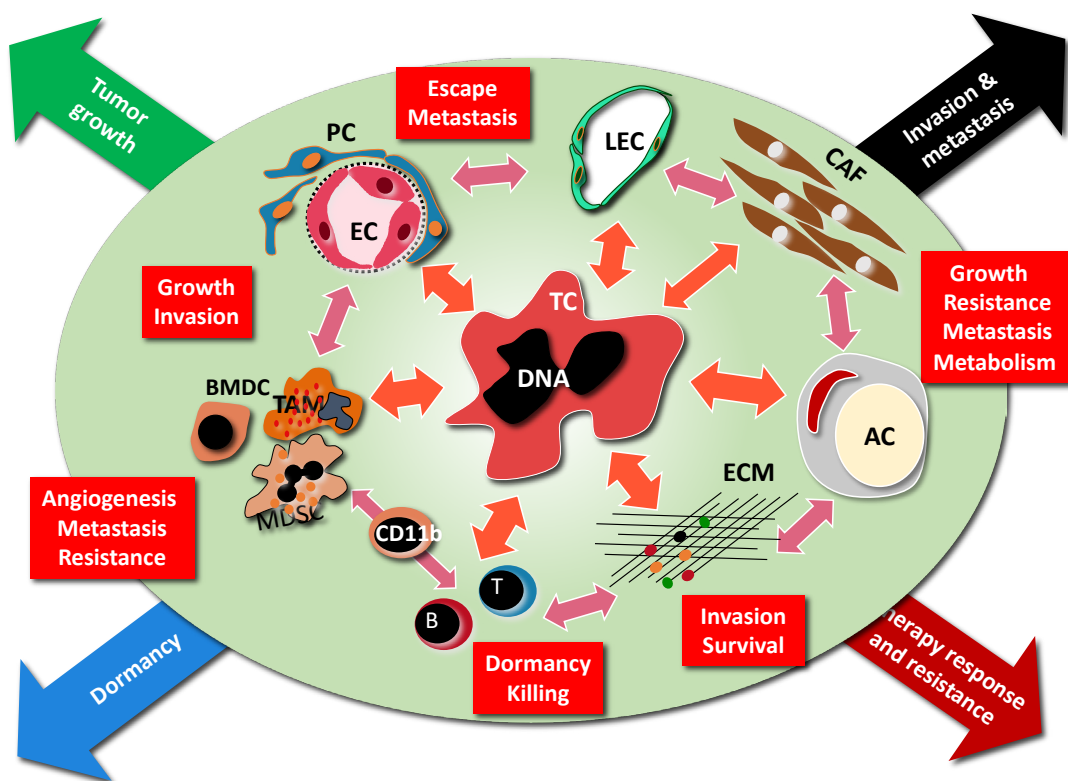


Fig. 1. **The tumor microenvironment.** Tumor cells orchestrate directly the modification of the microenvironment by attracting or activating a multitude of cells, including endothelial cells, carcinoma associated fibroblast, bone marrow-derived cells, and immune/inflammatory cells. Tumor cells can also modify the extracellular matrix. Most of these stromal modifications start early during tumor progression, often at the transition stage from pre-malignant to malignant lesions. Collectively, these events will contribute to determine the outcome of tumor progression: tumor growth, dormancy, invasion, metastasis and resistance to therapy.

Abbreviations: B, B lymphocyte; BMDC, bone marrow-derived cells; BV, blood vessel; CAF, carcinoma associated fibroblast; EC, endothelial cell; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; Gr, granulocyte; LEC, lymphatic endothelial cell; LV, lymphatic vessel; Mo, monocyte; MSC, mesenchymal stem cell; PC, pericyte; T, T lymphocyte; TAM, tumor associated monocyte/macrophage; TC, tumor cells.

Tumor-infiltrating immune/inflammatory cells play a dual role in cancer: on the one side they can repress tumor growth through the adaptive immune response (e.g. cytotoxic T cells, NK cells), yet, on the other side they can promote growth, invasion and metastasis through native immune cells (e.g. monocytes, macrophages). In addition, angiogenic vessels deliver nutrients to cancer cells, eliminate metabolic products, and provide an escape route for cancer cell dissemination. A multitude of inflammatory, survival and motility factors (e.g. VEGF, EGF, chemokines, prostaglandins) and altered extracellular matrix provide communication between host and tumor cells [3]. The tumor promoting events in the TME are focus of intense research by many groups including our (Fig. 2), clinicians, and pharmaceutical companies to explore novel diagnostic and therapeutic opportunities.

Projects in our laboratory focus on novel strategies for cancer detection and mechanisms of cancer progression and metastasis (Fig. 3). Our work is inspired by clinical questions and its overarching aim is to identify events and molecules with therapeutic, prognostic and predictive clinical relevance. Here we present some of the ongoing projects.

### Breast cancer metastasis

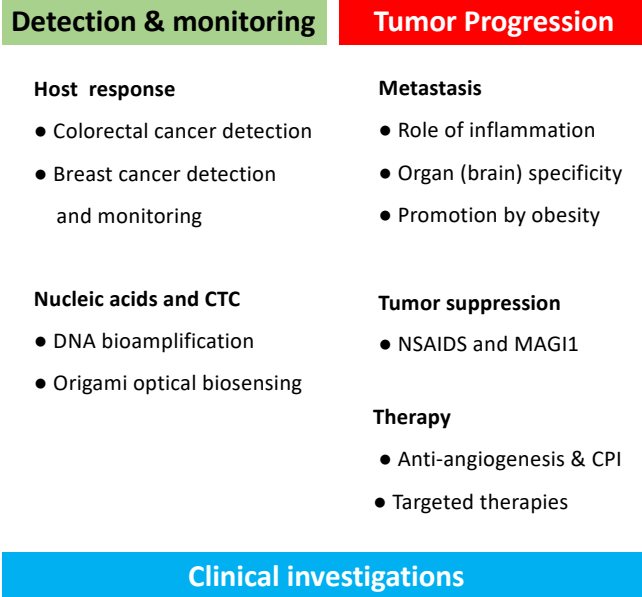
Cancer metastasis may be viewed as the ultimate outcome from the somatic evolution of cancer cells that have lost control over genomic integrity. The resulting genetic and cellular heterogeneity enables the selection for advantageous traits allowing malignant cells to overcome the diverse environmental obstacles encountered on their way to metastasis. Recent work, however,



**Fig. 2. The Experimental and Translational Oncology (ETO) Group at University of Fribourg.**

The main research interest of the group is the study of tumour-host interaction, tumour-associated inflammation and angiogenesis. Main achievements include the unraveling of the role of vascular integrins in mediating TNF cytotoxicity, COX-2-mediated angiogenesis, endothelial cell survival and vascular permeability. We identified Akt activation as a mediator of endothelial cell survival and of EMT in breast cancer. We characterized the role of CD11b+ leucocytes in angiogenesis, lymphangiogenesis, immunosuppression and tumor progression. We developed a blood-based test for detection of colorectal cancer based on altered gene expression in leukocytes now on the market (Colox®). Current and recent lab members include: Curzio Rüegg, Marie Tamm, Grégory Bieler, Mélanie Bousquenaud, Girieca Lorusso, Jimmy Stalin, Janine Wörthmüller, Samet Cokabey, Manuel Rodriguez-Perdigon, Sanam Peyvandi, Sarah Cattin, Ivana Domljanovic, Flavia Fico, Yunsha Zhang, Karima Shamaratova, Jeremy Kessler, Laetitia Genoud, Coralie Hoffmann, Oriana Coquoz, Melissa Rizza, Jhoana Scalogna, Ashot Sargsyan. For more information and publications see: [www3.unifr.ch/med/de/research/groups/ruegg/](http://www3.unifr.ch/med/de/research/groups/ruegg/)

**Research focus of the ETO Laboratory**



**Fig. 3. Topic of research at the experimental and translational oncology laboratory (ETO) at the University of Fribourg.**

We are investigating novel approaches for sensitive, minimally invasive cancer detection and monitoring (left) and mechanisms of tumor progression (right), notably mechanism of metastasis, and tumor suppression with the goal to identify novel mechanisms and molecules promoting metastasis to devise new therapeutic strategies. Most research focuses on breast cancer, and to a more limited extent, colorectal cancer. Whenever possible experimental results are validated by clinical investigations and patient data analyses (CPI, checkpoint inhibitors).

suggests that only a few additional «virulence» genes over those essential for primary tumor growth, occur on the way to metastasis [4]. Survival in the secondary tissue and colonization are emerging as the rate-limiting steps in metastasis. Disseminated cancer cells can «adapt» to the novel microenvironment through a combination of newly acquired traits and complementary cues provided by the surrounding tissue, particularly angiogenic and inflammatory/immune cells [3]. As there are no effective therapies to cure metastatic cancer, it is important to unravel mechanisms mediating metastatic dissemination, colonization and outgrowth.

We have identified the matricellular protein CCN1/CYR61 as potent mediator of metastasis through the VEGF-independent stimulation of tumor angiogenesis, promotion of cell invasion and survival via  $\alpha V$  and  $\beta 1$  integrins and suppression of anoikis through AMPK $\alpha$ -signaling [5, 6]. In preclinical and clinical studies, we

showed that anti-angiogenic therapy enhances the adaptive anti-tumor immune response and suppresses inflammatory tumor-promoting response [7, 8]. More recently, we showed that inhibition of host NOX1 with specific pharmacological inhibitors reduces tumor angiogenesis and enhances checkpoint inhibitor-based immunotherapy thereby eliciting a better therapeutic response [9].

**Breast cancer dormancy**

The kinetic of breast cancer relapse and metastasis is not linear in time but occurs with a bimodal distribution: a first peak 1-2 years after surgery and a second one 3-4 years later [10]. These observations suggested that disseminated cells do not grow continuously, but remain quiescent («dormant») for different periods of time, before resuming growth and forming metastases. Dormancy is clinically defined as the time elapsing between primary tumour removal and «late» relapse with no clinical evidence of diseases in the interval [11]. Mechanisms of cancer dormancy include cellular dormancy (i.e. cell cycle arrest), angiogenic dormancy (i.e. lack of angiogenesis) and immunological dormancy (i.e. cells kept in check by the immune system) [12]. Neoadjuvant and adjuvant chemotherapies provide survival benefits to breast cancer patients, in particular in ER<sup>-</sup> cancers, by reducing rates of recurrences. It is assumed that the benefits are due to the killing of residual cancer cells, however, there is no formal evidence for it. To test whether induction of dormancy may be involved in these effects we treated cancer cells with chemotherapy and characterized the behavior of surviving cells. We observed that treated and surviving cells had a sustained activation of the IRF7/IFN- $\beta$ /IFNAR pathway. IFN signaling twisted a tumor promoting, myelomonocyte-dominated immune response toward a CD4<sup>+</sup>/CD8<sup>+</sup> T cell dominated anti-tumor response keeping the disseminated cancer cells in check. Human data corroborated experimental results and showed that patients with a strong IFN response during chemotherapy had a better outcome [13]. These observations may open new opportunities to improve chemotherapy efficacy by stimulating IFN type I signaling in ER<sup>-</sup> breast cancers.

**Obesity-induced breast cancer relapses**

Obesity represents a risk factor not only for incidence of ER<sup>+</sup> breast cancer in post-menopausal women, but also for metastatic progression and resistance to therapy in all subsets regardless of the menopausal state [14]. The mechanisms involved in the latter effects remain largely elusive. To experimentally address this question, we developed mouse models of postmenopausal obesity, and demonstrated that in ER<sup>+</sup> breast cancer, obesity promotes the expansion of claudin low, TNBC-like metastasis-initiating cells. The pro-metastatic effect of obesity is associated with hypoxia and recruitment of inflammatory cells [15].

More recently, we have identified a specific population of inflammatory cells recruited to the TME of obese mice capable of inducing an immunosuppressive state. Inhibition of these cells prevented obesity-mediated metastasis (Bousquenaud et al., submitted). These results open new opportunity for personalized adjuvant therapy for obese breast cancer patients.

### Breast cancer metastasis to the brain

Brain metastasis is a late complication of metastatic breast cancer whose incidence is on the rise due to the longer survival achieved through a better control of metastatic disease in other organs [16]. Treatments for brain metastases (i.e. whole-brain radiation therapy, stereotactic radiosurgery, chemotherapy, targeted therapies), show limited efficacy reflected in the short survival time upon diagnosis [17]. Current models of brain metastasis bypass primary tumor development and do not recapitulate all the steps of the metastatic cascade as they occur in patients [18]. To address this problem, we have developed the first model of spontaneous breast cancer metastasis to the brain in immunocompetent mice. With this model, in combination with patient-derived data, we performed functional genomic screening to identify molecules mediating brain metastasis. We demonstrate that the colonization step in the brain is the rate limiting event in brain metastasis formation and identified several key molecular mediators, including two candidate targets for which there are clinically available drugs (Lorusso et al., in revision; Wyss et al., in revision). As two of these drugs inhibited progression of already established metastases, they may be further considered for clinical testing in the treatment of breast cancer patients presenting with brain metastases.

### NSAID and tumor suppression

Inflammation not only increases the risk of cancer incidence but also promotes metastasis through the production of motility factors (e.g. chemokines) and modification of the extracellular matrix, by secreting matrix modifying enzymes (e.g. MMPs) [2]. Accordingly, nonsteroidal anti-inflammatory drugs (NSAIDs) were shown to exert chemo-preventive effects in several cancers in particular of the colon [19], but also anti-tumor activities in adjuvant settings [20]. We have previously reported that COX-2 promotes tumor angiogenesis by regulating integrin function via PGE2 [21]. The long-term use of COXIBs, however, is not recommended to average risk individuals because of the elevated risk of potentially severe gastrointestinal and cardiovascular complications. In an effort to identify COXIBs-regulated tumor suppressors we performed a functional genomic screening and identified MAGI1 (MAGUK family member with inverted domain structure 1) as a new tumor suppressor in colorectal cancer. MAGI1 is a scaffolding protein that stabilizes

cadherin-mediated the cell-cell junctions. We showed that in colon cancer cells, MAGI1 suppresses Wnt signaling, induces a cohesive epithelial cell phenotype and decreases motility *in vitro*, and inhibits colorectal cancer growth, invasiveness and metastasis *in vivo* [22].

More recently, we showed that MAGI1 also acts tumor suppressive in ER<sup>+</sup>HER2<sup>-</sup> breast cancer [23]. Within this breast cancer subtype, high MAGI1 expression is associated with a better prognosis, while low MAGI1 expression correlates with higher histological grade, increased aggressiveness and worse prognosis. In ER<sup>+</sup> breast cancer cells, MAGI1 is upregulated by estrogen and contributes to ER signaling. MAGI1 downregulation in ER<sup>+</sup> cancer cells, impairs ER signaling, activates PI3K signaling and generates a more aggressive phenotype. Strikingly, MAGI1 is downregulated by COX2 activity and PGE2 while it is upregulated by COXIB. We are currently dissecting the link between inflammation, MAGI1 loss, inhibited ERS1 signaling and activation of the PI3K pathway that may contribute to resistance to hormonal therapy observed in a fraction of treated patients.

### New approaches for breast cancer detection and monitoring

In spite of a decrease in mortality by approximately 30% over the past 30 years, breast cancer (BC) remains the leading cause of cancer-related mortality for women in industrialized countries [24]. About one third of patients still die of the disease, due to the formation of metastases. In order to decrease breast cancer mortality, it is crucial to diagnose BC as early as possible, particularly in younger women, and to prevent or treat metastases effectively. Mammography is the gold-standard for the early detection of breast cancer, but in spite of its benefits in reducing BC specific mortality it has some important limitations [25]: limited specificity and sensitivity, risk of overdiagnosis, risk of inducing BC in patients with DNA repair defect (e.g. BRCA1/2), not recommended before the age of 50. Importantly, there are no validated specific tests to actively and specifically assess whether the disease is cured, dormant or progressing after initial therapy. Alternative methods are needed to improve early detection and monitoring. Liquid biopsies based on the detection of circulating tumor cells (CTC) or cell free tumor derived DNA (ctDNA) or RNA (e.g. miRNA) are being investigated but their clinical applicability is hampered by low sensitivity and technical hurdles (particularly for CTC detection) [26]. Thus, there is an unmet need for a more sensitive, specific, acceptable and economically viable method of BC early detection.

– **Detection of circulating tumor cells (CTC) by bio-inspired signal amplification.** CTC detection remains a challenging endeavor because of the low frequency of

these cells (1 cell per  $10^9$ - $10^{10}$  leucocytes) especially for clinical purposes [26]. We looked for inspiration in Nature to improve CTC detection. In events necessary to maintain homeostasis and respond to damages, eliciting signals are often rare or weak (e.g. few ligands or few receptors), therefore a cascade-like amplification of the incipient signals is engaged to generate vigorous responses (e.g. intracellular signaling, the inflammatory response and coagulation are based on this principle). We used a DNA hybridization chain reaction (HCR) [20] approach consisting of DNA oligonucleotide hairpins activated by an initiator oligonucleotide that will switch structure and self-assemble into amplification polymers. Fluorescent labels attached to the hairpins will amplify the signal of the proceeding reaction. We used this method to detect HER2<sup>+</sup> cancer cells by attaching the anti-HER2 antibody (trastuzumab) to the initiator oligonucleotide. This approach resulted in highly specific signal amplification of the bound DNA against HER2<sup>-</sup> cells and peripheral blood leukocytes [27]. While these results demonstrate the feasibility of the approach, the sensitivity, is still several orders of magnitude below the need for clinical detection and improvements are currently considered, in particular through the combination of plasmonic resonance-based detection.

– **Detection of nucleic acid by optical biosensing.** To detect cancer specific miRNAs with high sensitivity we are designing optical DNA origami biosensors [28]. Such biosensor consists of three rectangular layers of DNA helices connected with a hinge from the center allowing the opening of the layer at both sides. Layers are connected

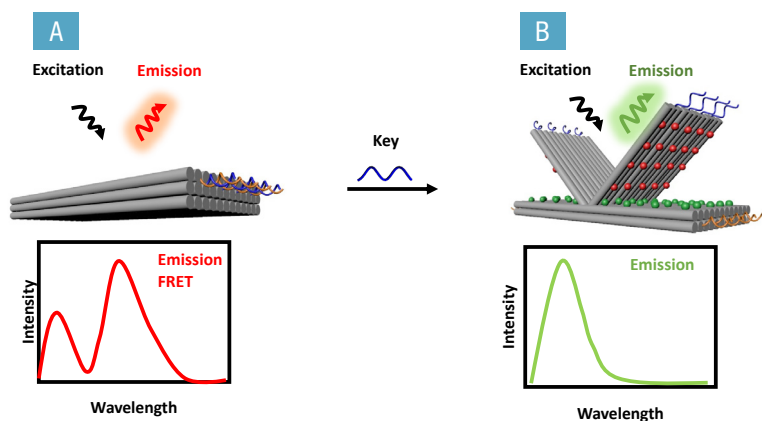
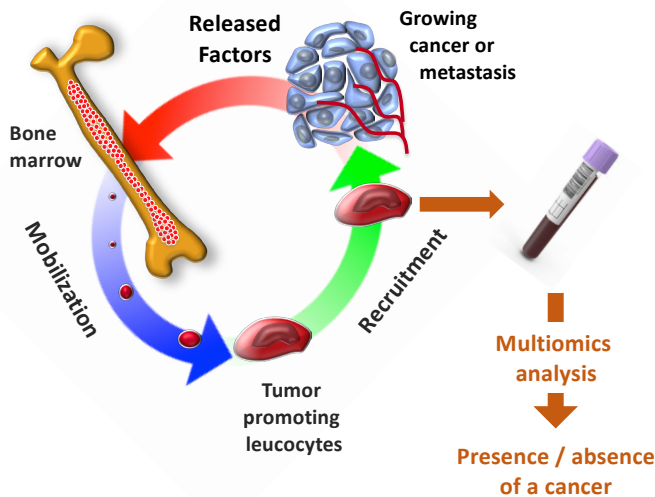


Fig. 4. Schematic representation of FRET-based detection mechanism of DNA origami biosensor. (A) In the absence of the target (key) the DNA origami is closed generating a FRET signal depicted in the spectrum below. (B) In the presence of the target (e.g. miRNA), the locks open resulting in change of FRET signal visible in the spectrum below (Image by I. Domljanovic).

with four locks on both sides to keep them in a closed state. Binding of miRNA to toehold causes opening of eth layers. In order to sense the binding of miRNAs, arrays of fluorophores are precisely positioned on top and middle layers. For detection we are using FRET and fluorescence quenching (Fig. 4). As a proof of concept, we are testing two breast cancer related miRNAs expressed in HER2<sup>+</sup> and triple negative breast cancer subtypes. Our results confirmed a difference in FRET efficiency between open and closed states of the biosensor. We are now optimizing quenching and FRET-based sensing mechanism in several conditions, as well as on single molecule level (Domljanovic, in preparation). We envision that DNA origami biosensors will offer an effective strategy for specific and sensitive detection of multiple disease related miRNAs or ctDNAs.

– **Exploiting the host response.** Growing primary tumors and metastases can mobilize myelomonocytic cells from the bone marrow which are then recruited at the tumor site to create an inflammatory-like microenvironment promoting tumor cell proliferation, survival, motility, angiogenesis and immune escape (Fig. 5) [2, 3]. By characterizing the transcriptome of circulating CD11b<sup>+</sup> cells, we have in the past defined a gene expression signature that lead to the development of a blood-based test for the non-invasive detection of advanced colorectal adenoma and cancer [29]. This test was further developed and validated in a large clinical study and is now on the market (COLOX®). More recently, we have performed preclinical and clinical studies using an advanced multi-omics analytical approach of circulating blood leukocytes and observed the appearance of bone marrow-derived cell populations associated with the presence of a primary or metastatic cancer (Cattin, submitted). We are now planning a multicentric case-control translational study to identify robust signatures to use in screening approach in complements to imaging-based methods and in breast cancer patients in remission after initial therapy to monitor disease progression. A test detecting relapses before metastases become symptomatic would allow adapting therapy when disease is still microscopic, before significant organ disruption and resistance have occurred. The need for such a test is intensified by the advent of effective second line therapies in recurrent cancers. This is best illustrated by the introduction of the PIK3CA inhibitor alpelisib in progressive ER<sup>+</sup> breast cancer. Combination of alpelisib with fulvestrant prolonged progression-free survival among patients with PIK3CA-mutated ER<sup>+</sup>/HER2<sup>-</sup> cancers that have relapsed under endocrine therapy [30]. Such a test would be integrated into current follow up protocols without disrupting ongoing practice.



**Fig. 5. Mobilized bone marrow leucocytes as biomarkers (and source thereof) to sense and monitor cancer.** Tumors and metastases mobilize myelomonocytic cells from the bone marrow through released factors. Mobilized cells are then recruited at the tumor site to promote tumor/metastasis growth. Characterization of these cells has revealed the appearance of rare CD11b+ cell populations and gene expression signatures associated with colorectal cancer. Similar observations were made in breast cancer.

### Conclusions and outlook

Our research has been inspired by clinically relevant questions with the long-term goal to identify mechanisms, molecules or events with therapeutic, prognostic and predictive implications. We have focused our work on two extremes of the broad spectrum of cancer: early detection and monitoring and metastatic disease. We believe that much remains to be done on both topics in order to decrease cancer-related mortality. We have been able to unravel mechanisms of breast cancer progression, metastasis and response to therapy and in particular the role of vascular and inflammatory cells. As the first laboratory at the University of Fribourg fully dedicated to experimental and translational cancer research after moving from Lausanne, we contributed to the raising visibility of Fribourg in this area of research. At the same time, our work would not have been possible without the collaborations that we have established over the years with many research laboratories and clinics in Switzerland and abroad, including: Department of Oncology CHUV (PD Dr. Zaman), Hôpital Fribourgeois (Prof. Betticher; Dr. B. Felley), IOSI Bellinzona (Prof. C. Sessa), IBR Bellinzona (Dr. Ugucconi), UNIL/SIB (Dr. M. Delorenzi), ZETUP (Dr. G. Fürstenberger), Clinica Luganese (Dr. A. Franzetti Pellanda), University of Fribourg (Prof. A. Fink, Prof. B. Rothen-Rüthishauser, Prof. M. Mayer, Prof. G. Acuna,

Prof. C. Szabo), University of Basel (Prof. G. Christofori), University of Geneva (Prof. B. Imhof, Prof. C. Bourquin), University of Bordeaux (Prof. A. Bikfalvi), ULB Brussels (Prof. C. Sotiriou), KLU Leuven (Prof. C. Desmedt) and more. For these collaborations we are very thankful. In the coming years we will further focus on clinically-oriented projects, in particular on the identification and validation of blood biomarker for (breast) cancer detection and monitoring, the translation of results on metastatic progression toward clinical testing, and the development of nanotools for the sensitive detection of miRNA and mRNA. We are open to and welcome further collaborations, as we believe that research in general, and cancer research in particular, vastly profit from sharing ideas, competences and resources.

### Acknowledgments

I would like to thank all past and present member of the laboratory (Fig. 2) for their excellent work, motivation and endurance on sometimes difficult projects. I also would like to thank all external collaborators, in particular clinical colleagues and patients, without whom translational projects would not have been possible. I am also thankful to funding agencies and sponsors that have supported our research throughout the years: Swiss Cancer League, Swiss National Science Foundation, CTI/Innosuisse, NCCR Molecular Oncology, NCCR Bioinspired Materials, Medic Foundation, ISREC Foundation, Swiss Heart Foundation, Stiftung zur Krebsbekämpfung, Leenards Foundation, Fondazione San Salvatore, Gebert Rüt Stiftung, Roche Research Foundation, Novartis Foundation, 3R Foundation, EU-FP7 programs (Cooperation Health TUMIC; MSC Smart; MSC, Smarter), the University of Fribourg, the Multidisciplinary Oncology Center at CHUV, Lausanne.

### References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 144: 646-674, 2011.
2. Lorusso G, Ruegg C. The tumor microenvironment and its contribution to tumor evolution toward metastasis. *Histochem Cell Biol* 130: 1091-1103, 2008.
3. Sleeman JP, Christofori G, Fodde R, et al. Concepts of metastasis in flux: The stromal progression model. *Semin Cancer Biol* 2012; in press.
4. Chiang AC, Massague J. Molecular basis of metastasis. *N Engl J Med* 359: 2814-2823, 2008.
5. Huang YT, Lan Q, Lorusso G, Duffey N, Ruegg C. The matrix-cellular protein CYR61 promotes breast cancer lung metastasis by facilitating tumor cell extravasation and suppressing anoikis. *Oncotarget* 8: 9200-9215, 2017.
6. Monnier Y, Farmer P, Bieler G, et al. CYR61 and alphaVbeta5 integrin cooperate to promote invasion and metastasis of tumors growing in preirradiated stroma. *Cancer Res* 68: 7323-7331, 2008.

7. Secondini C, Coquoz O, Spagnuolo L, et al. Arginase inhibition suppresses lung metastasis in the 4T1 breast cancer model independently of the immunomodulatory and anti-metastatic effects of VEGFR-2 blockade. *Oncoimmunology* 6: e1316437, 2017.
8. Cattin S, Fellay B, Pradervand S, et al. Bevacizumab specifically decreases elevated levels of circulating KIT+CD11b+ cells and IL-10 in metastatic breast cancer patients. *Oncotarget* 7: 11137-11150, 2016.
9. Stalin J, Garrido-Urbani S, Heitz F, et al. Inhibition of host NOX1 blocks tumor growth and enhances checkpoint inhibitor-based immunotherapy. *Life Sci Alliance* 2: e201800265, 2019.
10. Demicheli R. Tumour dormancy: findings and hypotheses from clinical research on breast cancer. *Semin Cancer Biol* 11: 297-306, 2001.
11. Demicheli R, Retsky MW, Hrushesky WJ, Baum M. Tumor dormancy and surgery-driven interruption of dormancy in breast cancer: learning from failures. *Nat Clin Pract Oncol* 4: 699-710, 2007.
12. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. *Nat Rev Cancer* 7: 834-846, 2007.
13. Lan Q, Peyvandi S, Duffey N, et al. Type I interferon/IRF7 axis instigates chemotherapy-induced immunological dormancy in breast cancer. *Oncogene* 38: 2814-2829, 2019.
14. Widschwendter P, Friedl TW, Schwentner L, et al. The influence of obesity on survival in early, high-risk breast cancer: results from the randomized SUCCESS A trial. *Breast Cancer Res* 17: 129, 2015.
15. Bousquenaud M, Fico F, Solinas G, Ruegg C, Santamaria-Martinez A. Obesity promotes the expansion of metastasis-initiating cells in breast cancer. *Breast Cancer Res* 20: 104, 2018.
16. Takei H, Rouah E, Ishida Y. Brain metastasis: clinical characteristics, pathological findings and molecular subtyping for therapeutic implications. *Brain Tumor Pathol* 33: 1-12, 2016.
17. Franchino F, Ruda R, Soffiati R. Mechanisms and Therapy for Cancer Metastasis to the Brain. *Front Oncol* 8: 161, 2018.
18. Bos PD, Nguyen DX, Massague J. Modeling metastasis in the mouse. *Curr Opin Pharmacol* 10: 571-577, 2010.
19. Moris D, Kontos M, Spartalis E, Fentiman IS. The Role of NSAIDs in Breast Cancer Prevention and Relapse: Current Evidence and Future Perspectives. *Breast Care (Basel)* 11: 339-344, 2016.
20. Choi HM, Beck VA, Pierce NA. Next-generation in situ hybridization chain reaction: higher gain, lower cost, greater durability. *ACS Nano* 8: 4284-4294, 2014.
21. Dormond O, Foletti A, Paroz C, Ruegg C. NSAIDs inhibit alpha V beta 3 integrin-mediated and Cdc42/Rac-dependent endothelial-cell spreading, migration and angiogenesis. *Nat Med* 7:1041-1047, 2001.
22. Zaric J, Ruegg C. Integrin-mediated adhesion and soluble ligand binding stabilize COX-2 protein levels in endothelial cells by inducing expression and preventing degradation. *J Biol Chem* 280: 1077-1085, 2005.
23. Alday-Parejo B, Richard F, Worthmuller J, et al. MAGI1, a New Potential Tumor Suppressor Gene in Estrogen Receptor Positive Breast Cancer. *Cancers (Basel)* 12: 223, 2020. doi: 10.3390/cancers12010223.
24. Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2012. *Ann Oncol* 23: 1044-1052, 2012.
25. van den Ende C, Oordt-Speets AM, Vroiling H, van Agt HME. Benefits and harms of breast cancer screening with mammography in women aged 40-49 years: A systematic review. *Int J Cancer* 141: 1295-1306, 2017.
26. Alix-Panabieres C, Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. *Cancer Discov* 6: 479-491, 2016.
27. Rafiee SD, Kocabay S, Mayer M, List J, Ruegg C. Detection of HER2(+) Breast Cancer Cells using Bioinspired DNA-Based Signal Amplification. *ChemMedChem* 15: 661-666, 2020.
28. Loretan M, Domljanovic I, Lakatos M, Ruegg C, Acuna GP. DNA Origami as Emerging Technology for the Engineering of Fluorescent and Plasmonic-Based Biosensors. *Materials (Basel)* 13: 2185, 2020. doi.org/10.3390/ma13092185.
29. Ciaroni L, Ehrensberger SH, Imaizumi N, et al. Development and Clinical Validation of a Blood Test Based on 29-Gene Expression for Early Detection of Colorectal Cancer. *Clin Cancer Res* 22: 4604-4611, 2016.
30. Andre F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. *N Engl J Med* 380: 1929-1940, 2019.

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