MOHAMED BENTIRES-ALJ

CURRICULUM VITAE

Family name: Bentires-Alj
First name: Mohamed
Date of birth 18.07.1972
Place of birth: Casablanca (Morocco)
Citizenships: Belgian and Moroccan

Professor of Experimental Surgical Oncology
Tumor Heterogeneity, Metastasis and Resistance
Department of Biomedicine
University of Basel / University Hospital Basel
Lab 306, Hebelstrasse 20, CH-4031 Basel/Switzerland
E-mail: m.bentires-alj@unibas.ch; +41 (0) 61 26 53 313
URL for web site: https://bentireslab.org/

Education

1996	Pharmaceutical Sciences, University of Liège, Belgium
2001	Ph.D. *(summa cum laude)* in Pharmaceutical Sciences, University of Liège, Belgium

Current Positions

2017–	Chair of the Swiss Personalized Oncology Chair of the Basel personalized health "cancer cluster"
2016–	Professor of experimental surgical oncology, University of Basel

Previous Positions

2013–2016	Senior staff scientist at the Friedrich Miescher Institute, Basel, Switzerland
2006–2013	Junior group leader at the Friedrich Miescher Institute, Basel, Switzerland
2004–2006	Research Assistant, National Fund for Scientific Research (FNRS), Belgium
2001–2006	Postdoctoral fellow: Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, USA

Approved research projects (since 2015)

2021–2024:	MSCA ITN project EVOMET: Horizon 2020
2020–present	Board member of IABCR: International Association for Breast Cancer Research
2019–2023:	Swiss National Foundation (SNF)
2019–2021:	Krebsliga Beider Basel
2019–2022:	OncoSuisse grant. Swiss Cancer League
2018–2021:	Swiss Personalized Health Network (SPHN) driver project
2016–2021:	European Research Council (ERC) advanced investigator grant
2015–2018:	Swiss Initiative in Systems Biology: SystemsX

Prizes, fellowships, distinguished memberships (since 2010)

2016	Elected European Molecular Biology Organization (EMBO) member
	European Research Council (ERC) Advanced grant
2015	American Association for Cancer Research (AACR): Outstanding Investigator in Breast Cancer Research Award
2014	Robert Wenner Award of the Swiss cancer league
	S. G. Komen for the Cure, European Association for Cancer Research (EACR) Award
	Proffered Paper Award, EACR23
	Novartis Select Award
	Chair of the Mammary Gland Biology Gordon Research Conference
2013	Novartis Select Award
2012	Dora-Seif Prize for Cancer Research, University of Basel, Switzerland
2010	European Research Council (ERC) young investigator starting grant

Board memberships

- Elected board member of the Metastasis Research Society (www.metastasis-research.org) (since 2020).

- Board member of IABCR: International Association for Breast Cancer Research (since 2020).
- Journal of Mammary Gland Biology and Neoplasia (since 2012) – *Editorial board*
- Breast Cancer Research (since 2008) – Associate *Editor*
- Cancer Research (2013-2019) – *Editorial board*
- Krebsliga Beider Basel (since 2010) – *Scientific board*
- European Network for Breast Development and Cancer (www.enbdc.org) (since 2008) – *Founder and President*
- Basel Breast Consortium (BBC) (www.BaselBC.org) (since 2014) – *Co-founder and Coordinator*
- Translational working group EU-Life (2013–2017) – *Committee Member*
- Medalis University of Strasbourg, France (since 2015) – *Scientific advisory board*
- F.R.S.-FNRS, Belgium (2015-2019) – *Scientific Commission*
- Breast Cancer Now Toby Robins Research Centre at the ICR, London – *Scientific advisory board*
- Scientific Committee of the Dora-Seif Stiftung (since 2020)

Organization of conferences (since 2014)

2019	Personalized oncology 2019, Basel, Switzerland
2018	Co-chair of the EuroPDX meeting, Weggis, Switzerland
2018	International PhD course on Frontiers in Metastasis, Basel, Switzerland
2016 – 2020	Basel Breast consortium annual meeting on personalized breast cancer treatment, Basel, Switzerland
2016	Scientific committee of the EuroPDX meeting, Weggis, Switzerland
	EU-LIFE Tumour Microenvironment and Metastasis PhD course, Copenhagen, Denmark
2015	Member of the scientific committee of the 2015 LS2 meeting, Zurich, Switzerland
2014	Chair of the Mammary Gland Biology Gordon Research Conference, Tuscany, Italy
	Organizing committee of the Targeting the kinome III meeting, Basel, Switzerland

Patents

1 Combination of a phosphoinositide 3-kinase inhibitor and a modulator of the Janus Kinase 2 – Signal Transducer and Activator of Transcription 5 pathway, FMI-087/00EP
2 Interleukin-8 and breast cancer, FMI-090/00EP
3 PTPN11 and tumor-initiating cells, FMI-077/00WO
4 Culture medium suitable for the culture of undifferentiated cells, FMI-082/00WO
5 CDCP1 and breast cancer, FMI-088/00EP
6 PTPN11 and triple-negative breast cancer, FMI-083/00WO
7 Roles of RHAU in cancer (with Yoshi Nagamine), FMI-061/00WO
8 LATS and breast cancer, FMI-EP14186104.7
9 Treating cancer by modulating RNA helicases, US Patent App. 13/120,353

SELECTED PUBLICATIONS

Glucocorticoids promote breast cancer metastasis.
Obradović MMS, Hamelin B, Manevski N, Couto JP, Sethi A, Coissieux A, Münst S, Okamoto R, Kohler H, Schmidt A, **Bentires-Alj M**
Nature, 567(7749):540-54 (2019)

The Hippo kinases LATS1/2 control human breast cell fate via crosstalk with ERα.
Britschgi A, Duss S, Kim S, Couto JP, Brinkhaus H, De Silva D, Mertz KD, Kaup D, Varga Z, Voshol H, Vissieres A, Leroy C, Roloff T, Stadler M, Koren S, Scheel C, Miraglia L., Orth P.A., Bonamy G.M.C., Reddy V, **Bentires-Alj M**
Nature, 541(7638):541-545 (2017)

PIK3CA^{H1047R} induces multipotency and multi-lineage mammary tumors.
Koren S, Reavie L, Silva J, De Silva D., Stadler M., Roloff T., Britschgi A, Eichlisberger T., Kohler H., Aina O., Cardiff RD, **Bentires-Alj M**
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Tyrosine phosphatase SHP2 increases cell motility in triple negative breast cancer via activation of SRC-family kinases.
Sausgruber N, Coissieux MM, Britschgi A, Wyckoff J, Aceto N, Leroy C, Voshol H, Bonenfant D, **Bentires-Alj M**
Oncogene, 34(17):2272-8 (2015)

Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis.
Bonapace L, Coissieux MM, Wyckoff J, Mertz K, Varga Z, Junt T, and **Bentires-Alj M**
Nature, 515(7525):130-3 (2014)

Parity induces differentiation and reduces Wnt/Notch signaling ratio and proliferation potential of basal stem/progenitor cells isolated from mouse mammary epithelium.
Meier-Abt F, Milani E, Roloff T, Brinkhaus H, Duss S, Meyer DS, Klebba I, Balwierz P, van Nimwegen E, **Bentires-Alj M**
Breast Cancer Research, 15(2): R36 (2013).

The calcium activated chloride channel ANO1 promotes breast cancer progression by activating EGFR- and CAMK-signaling.
Britschgi A, Bill A, Brinkhaus H, Rothwell C, Clay I, Duss S, Rebhan M, Raman P, Guy C, Wetzel K, George E, Oana Popa M, Lilley S, Choudhury H, Gosling M, Wang L, Fitzgerald S, Borawski J, Baffoe J, Labow M, Gaither LA, **Bentires-Alj M**
PNAS plus, 110 (11) 1026-34 (2013).

JAK2/STAT5 inhibition circumvents resistance to PI3K/mTOR blockade, providing a rationale for co-targeting these pathways in metastatic breast cancer.
Britschgi A, Andraos R, Brinkhaus H, Klebba I, Romanet V, Müller U, Murakami M, Radimerski T, **Bentires-Alj M**
Cancer Cell, 22(6):796-811 (2012)

Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop.
Aceto N, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, Confalonieri S, Hu G, Balwierz P, Pachkov M, Elledge SJ, van Nimwegen E, Stadler MB, **Bentires-Alj M**
Nature Medicine, 18(4): 529-37 (2012)

Luminal expression of mutant *PIK3CA* in the Mammary Gland Induces Heterogeneous Tumors.
Meyer D, Brinkhaus H, Muller U, Muller M, Cardiff RD, **Bentires-Alj M**
Cancer Research 71(13):4344-51 (2011)

BREAST TUMOR HETEROGENEITY, METASTASIS, AND THERAPY RESISTANCE IN THE ERA OF PERSONALIZED MEDICINE

Mohamed Bentires-Alj [1]

Summary

Breast cancer is the second leading cause of cancer death in women and 2.1 million new patients are diagnosed with breast cancer annually. While 98% of patients survive 5 years or more after diagnosis of a localized (confined to the primary site) breast cancer, this number drops to 15–25% if the cancer has metastasized to distant organs. Thus, curing metastatic breast cancer is clearly an unmet medical need. The cellular and biochemical mechanisms that lead to drug-resistant metastases remain largely unknown and their identification has been my primary goal for the last 20 years. New therapies are likely to result from a more thorough understanding of cancer as a systemic disease involving both genomic alteration of cancer cells and dynamic crosstalk between cancer cells and the tumor microenvironment (e.g., immune cells). The thread connecting the research topics in my lab is tumor heterogeneity. We assess fundamental mechanisms that influence normal and neoplastic breast stem cells, metastasis, and resistance to targeted therapies at the molecular, cellular, and whole organism levels. These interdisciplinary projects seek to leverage a mechanistic insight into personalized therapy, which is a recent focus of the translational research that we pursue in close collaboration with clinicians from the University Hospital Basel (USB) (Figure 1) (www.bentireslab.org). In this review, I summarize a selection of our basic and translational research findings, discuss some of our ongoing projects, and highlight our efforts in personalized medicine in Basel, in Switzerland, and worldwide.

[1] Department of Biomedicine, Department of Surgery, University Hospital Basel, University of Basel, Switzerland

Introduction

"If I have seen further it is by standing on the shoulders of Giants", wrote Isaac Newton.

Observation and research in mammary gland biology and cancer over the previous centuries have laid the foundation for our current understanding of this fascinating organ. But, despite the spectacular breakthroughs in our understanding of its pathophysiology and the corresponding clinical advances made by several key figures in our field (for an oral history of our field, see https://enbdc.org/interviews/), breast cancer is still a source of worry and distress for patients.

The mammary gland. The mammary gland is an epidermal appendage that evolved with mammals around 300 million years ago, plausibly from apocrine sweat glands[1]. The branched ductal-alveolar tree making up the

Figure 1. *Research areas within the Bentires-Alj lab*

mammary gland is surrounded by a basement membrane and stromal cells, and is composed of hierarchically organized cell types that contribute to tissue homeostasis. Two major cell lineages, organized in a bi-layered structure, constitute the mammary gland epithelium. The luminal layer lining the ducts and the alveoli is composed of cells that express keratin 8/18 (K8/18) and/or estrogen and/or progesterone receptor (ER/PR). The myoepithelial layer with a basal location is composed of cells that express K5/14 and/or smooth muscle actin (SMA) and/or p63[2,3]. Distinct mammary epithelial cell subpopulations can be isolated from mouse mammary glands by fluorescent-activated cell sorting (FACS) using specific cell-surface markers[4–7]. Inducible genetic lineage tracing, which permits targeted expression of a fluorescent reporter in a given cell and its progeny, has identified unipotent luminal K8/18-positive and basal K5/14- and Lgr5- (leucine-rich-repeat-containing G-protein-coupled receptor 5) positive stem cells after birth[8] (Figure 2). Multipotent cells that generate both the luminal and basal lineages are present in the mouse embryonic mammary gland[8,9]. Breast cancer originates from mammary epithelial cells and a key issue in breast cancer biology is the effect of genomic lesions in specific mammary cell lineages on tumor subtype, heterogeneity, and progression.

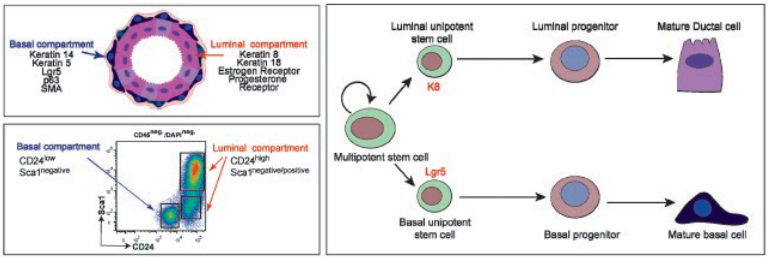

Figure 2. Upper left: *Schematic of a cross-section of a mammary gland duct showing the two major cell lineages that constitute the mammary gland epithelium.* Lower left: *FACS strategy for sorting different mammary subpopulations.* Right: *Mammary gland hierarchy. K8: keratin8. Lgr5: leucine-rich-repeat-containing G-protein-coupled receptor 5*

Breast cancer. Worldwide, nearly 650,000 lives are lost to breast cancer annually, the vast majority due to drug-resistant metastases[10–12]. Breast cancer is a heterogeneous disease that progresses to metastases of lung,

bone, liver, and/or brain, with fatal complications[13–16]. Molecular profiling of primary tumors has identified six intrinsic breast cancer subtypes: normal-like, luminal A, luminal B, HER2-enriched, claudin-low, and basal-like breast cancer[17–19]. Each subtype has a characteristic disease progression and clinical outcome[18,20]. Integrated genome-wide analyses of DNA copy number, RNA expression, and exome sequencing of human breast tumors has revealed a multitude of alterations within cancer cells[21–25].

Although such findings in the last decades have improved our understanding of molecular mechanisms underlying the disease, we still lack effective targeted therapies for many aggressive breast cancer subtypes. In the clinic, three main biomarkers are used to define pharmacological treatment: estrogen receptor α (ERα), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Expression of ERα and/or PR is typically associated with luminal A and B breast cancers, which are frequently responsive to endocrine therapy. Targeted therapies are available (e.g., Trastuzumab) for HER2-positive breast cancer. Tumors lacking expression of all three biomarkers are commonly referred to as triple-negative breast cancer (TNBC) and patients are treated with chemotherapy[26,27].

Tumor heterogeneity. Long before the era of molecular biology, pathologists observed breast cancer heterogeneity in tumors from different patients (intertumoral) and within the same tumor (intratumoral)[13,28–30]. Several factors are thought to contribute to breast tumor heterogeneity: the differentiation state of the cell in which the cancer originates, genetic and epigenetic oncogenic alterations, stochastic events, the tumor microenvironment, and/or a therapy. Notably, a single tumor genotype can have multiple phenotypic manifestations, indicating that cancer phenotype may also result from non-genetic determinants[13,31,32]. Non-genetic mechanisms have been shown to influence normal and neoplastic tissue stem-cell hierarchy, raising the possibility that they may also generate hierarchically organized breast tumors with a self-renewing cancer stem-cell subpopulation. The genetic evolution and the cancer stem-cell models are not necessarily mutually exclusive and a unifying model has been proposed[33].

Clinical implications. Altogether these observations indicate the existence of cancer cells with different biological properties (e.g., self-renewal, proliferation, survival, metastatic capability, response to therapy) within the same tumor. Cancer progression seems to follow a Darwinian evolution model and the genetic and epigenetic alterations in cancer cells result in subclones with different phenotypes that are subjects of selective evolution. The clinical implications of tumor heterogeneity and selective evolution are paramount. Because of region-to-region and cell-to-cell heterogeneity, biopsy of a small tumor region may confuse prognostic and predictive biomarkers and result in therapy failure.

1. Examples from previous studies from our laboratory:

1.1. Breast tumor heterogeneity: the importance of the cell-of-origin of breast cancer

Breast cancer is a heterogeneous disease and, besides the nature and number of genomic-transforming events and microenvironmental factors, the differentiation state of the cell-of-origin of cancer also determines the phenotype, tumorigenicity, and metastatic potential of this malignancy.

First, we addressed the impact of an activated phosphoinositide 3-kinase (PI3K) pathway on fate conversion in different cancer cells-of-origin and thus their contribution to tumor heterogeneity. The PI3K pathway is a central regulator of diverse normal cellular functions. It is one of the most essential pathways producing hallmarks of cancer[34]. PI3Ks are lipid kinases that phosphorylate phosphoinositides, leading to the activation of downstream kinases that influence key physiological processes such as metabolism, proliferation, cell growth, survival, and motility. It is estimated that up to 70% of breast cancers feature a hyperactive PI3K cascade[35,36]. Given the key effects of the PI3K pathway in solid cancers, important drug discovery programs have yielded a variety of compounds that efficiently target this pathway and are currently being evaluated in clinical trials. Notably, Alpelisib (BYL719), an alpha-specific PI3K inhibitor, was approved by the FDA for use in combination with the endocrine therapy fulvestrant for treatment of hormone receptor-positive and HER2-negative breast cancer. The gene *PIK3CA* encodes the PI3K catalytic subunit p110α and its amplification and/or mutation is associated with several kinds of human solid tumors. Activating somatic mutations

in *PIK3CA* are present in ~30% of human breast cancers at all stages. In 47% of these cases, mutations occur in the kinase domain, the most frequent being H1047R in exon 20. A hyperactive PI3K pathway results in cancer cells with a competitive advantage because of a decrease in cell death and increases in cell proliferation, migration, invasion, metabolism, angiogenesis, and resistance to chemotherapy[37,38]. We and others have shown that inducible expression of *PIK3CA* mutants induces mammary tumors in mice[34,38–41].

Using *in situ* genetic lineage tracing and limiting dilution transplantation, as well as mouse models of *PIK3CA*^{H1047R} generated in our lab, we have unraveled the potential of *PIK3CA*^{H1047R} to induce multipotency during tumorigenesis in the mammary gland (Figure 3). Our results and those of others define a key effect of *PIK3CA*^{H1047R} on mammary cell fate in the pre-neoplastic mammary gland[42,43]. We show that the cell-of-origin of *PIK3CA*^{H1047R} tumors dictates their malignancy, thus revealing a mechanism underlying tumor heterogeneity and aggressiveness[42].

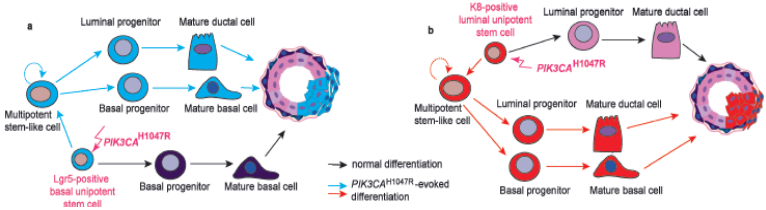

Figure 3. *The effect of* PIK3CA^{H1047R} *expression in basal Lgr5-* ***(a)*** *and luminal K8-positive* ***(b)*** *lineage-restricted mouse mammary cells. Mammary cells expressing* PIK3CA^{H1047R} *dedifferentiate into a multipotent stem-like state from which they further differentiate into the basal and luminal cell lineage. Expression of* PIK3CA^{H1047R} *in Lgr5-positive cells led mostly to benign but in K8-positive cells mostly to malignant mammary tumors.* Black arrows *indicate the differentiation potential of Lgr5- and K8-positive cells under physiological conditions*

Second, we used a high-content confocal image-based shRNA screen for tumor suppressors regulating human breast cell fate. By studying primary human breast epithelial cells, we have discovered that ablation of the Hippo kinases large tumor suppressors (LATS) 1 and 2 promotes luminal fate and increases the number of breast bipotent and luminal progen-

itors, the proposed cell-of-origin of most human breast cancers. Mechanistically, we discovered a crosstalk between Hippo and ERα signaling. In the presence of LATS, ERα was targeted for ubiquitination and Ddb1–cullin 4-associated-factor 1 (DCAF1)-dependent proteasomal degradation. Removal of LATS in ERα-positive cancer cells reduced their sensitivity to the widely used selective ER downregulator fulvestrant. Our findings reveal a non-canonical (i.e., YAP/TAZ-independent) effect of LATS in the regulation of human breast cell fate[44].

1.2. Breast tumor heterogeneity and progression to metastasis
Glucocorticoids promote breast cancer metastasis

A thorough understanding of the molecular and cellular mechanisms underlying both intra-patient breast tumor heterogeneity and metastasis is crucial for the success of personalized cancer therapy. Intra-patient tumor heterogeneity describes a poorly understood phenomenon during malignant progression by which cancer cells and patients themselves undergo genetic and epigenetic as well as hormonal and immunological changes. Phenotypic changes in cancer cells are a consequence of selection and adaptation that may result in cancer growth at distant sites years after primary tumor diagnosis and removal. Tumor heterogeneity is an obstacle to treatment, spawning divergence in diagnostic markers between primary tumors and matched metastases that may lead to inadequate treatment. We have recently shown cancer site-specific phenotypes and increased glucocorticoid receptor (GR) activity in distant metastases using transcriptional profiling of triple-negative breast tumors and matched metastases. GR mediates the effects of the stress hormones and synthetic derivatives (i.e., dexamethasone) used widely in the clinic as anti-inflammatory and immunosuppressive agents. We show that increase in stress hormones during breast cancer progression results in GR activation at distant metastatic sites, increased colonization, and ultimately reduced survival. To address the molecular mechanism underlying these observations, we performed transcriptome profiling, proteomics, and phosphoproteomics studies. The results implicated GR in the activation of multiple processes in metastasis and in increased expression of kinase ROR1, which correlates with shorter patient survival.

We also find that the stress hormone pathway is an effective inducer of colonization and the death of the animals, and that ROR1 knockdown counteracts this deleterious effect of GR activation and prolonged survival in preclinical models. The data also reveal that GR activation decreases the efficacy of the widely used chemotherapy paclitaxel. Corticosteroids such as dexamethasone are widely used in the treatment of breast cancer to combat side-effects of chemotherapy and to treat symptoms related to advanced cancer. Given that cancer cell dissemination has already occurred by the time of primary tumor surgical resection in a substantial number of breast cancer patients[14,29], and that GR activation fosters colonization at the distant sites, our results call for caution when administering corticosteroids to patients with cancer-related complications[45].

Targeting SHP2 in breast cancer

The first *bona fide* protein tyrosine phosphatase proto-oncogene is the Src-homology 2 domain-containing phosphatase SHP2. A ubiquitously expressed protein, SHP2 transduces mitogenic, pro-survival, cell fate, and/or pro-migratory signals from almost all growth factor, cytokine and extracellular matrix receptors[46]. SHP2 is required for full activation of the ERK/MAPK pathway downstream of most of these receptors. In cancer, SHP2 is hyperactivated either by mutations or downstream of oncogenes. We and others have shown that these mutations occur at various incidences in myeloid malignancies but rarely in solid cancers[47–49]. SHP2 is also activated downstream of oncogenes upon binding to phosphorylated proteins[50]. Whereas we found no mutations of SHP2 in human breast cancer samples[49], we initially discovered that SHP2 is required for GAB2-evoked increased proliferation and invasiveness in breast cancer models[51]. We have demonstrated a fundamental effect of SHP2 on breast tumor maintenance and progression. SHP2 knockdown eradicated breast tumor-initiating cells *in vitro* and in xenografts. Serial limiting dilution transplantation experiments over three passages revealed that SHP2 knockdown decreases tumor seeding and propagation. SHP2 activated c-Myc and ZEB1, which resulted in repression of let-7 microRNA and the expression of a set of "SHP2 signature" genes found to be co-activated in human primary breast tumors. Using phosphoproteomics and intravital imaging, we found that SHP2 also activates c-SRC, leading to an

increase in cancer cell motility. Our studies provided new insights into signaling cascades that regulate neoplastic breast stem cells and a rationale for targeting SHP2 in breast cancer. SHP2 inhibitors are currently being evaluated in clinical trials[52–54].

1.3. Resistance to therapy, therapy for resistance
Inhibition of PI3K and tumor heterogeneity

Selection of specific tumor clones or activation of a bypass pathway upon exposure of cancer cells to treatment also results in tumor heterogeneity. We discovered a JAK2/STAT5-evoked positive feedback loop that dampens the efficacy of dual PI3K/mTOR inhibition in triple-negative breast cancer. Mechanistically, PI3K/mTOR inhibition increased IRS1-dependent activation of JAK2/STAT5 and secretion of IL8. Genetic or pharmacological inhibition of JAK2 abrogated this feedback loop, and combined PI3K/mTOR and JAK2 inhibition synergistically reduced cancer cell number, decreased tumor seeding and metastasis, and increased overall survival of the animals. Our results provide a rationale for combined targeting of the PI3K/mTOR and IL8/JAK2/STAT5 pathways in triple-negative breast cancer[55].

In luminal breast cancer cells, we found an increase in IGF1R, IRS1/IRS2 and p85 phosphorylation in cancer cells resistant to the p110α isoform-selective inhibitor BYL719. Co-immunoprecipitation experiments identified an IGF1R/IRS/p85/p110β complex that causes the activation of AKT/mTOR/S6K and stifles the effects of BYL719. Pharmacological inhibition of members of this complex reduced mTOR/S6K activation and restored sensitivity to BYL719. Our study demonstrates that p110β confers resistance to BYL719 in *PIK3CA* mutant breast cancers. This provides a rationale for the combined targeting of p110α with IGF1R or p110β in patients with breast tumors harboring PIK3CA mutations[56,57].

Halting blockade of the innate immune system results in cancer heterogeneity

We have discovered a paradoxical effect of the CC chemokine ligand 2 (CCL2) in metastatic breast cancer. Secretion of CCL2 by mammary tumors recruits CCR2-expressing inflammatory monocytes to primary tu-

mors and metastatic sites, and CCL2 neutralization in mice inhibits metastasis by retaining monocytes in the bone marrow. Surprisingly, interruption of CCL2 inhibition leads to an overshoot of metastases and accelerates death. This is the result of monocyte release from the bone marrow, enhancement of cancer cell mobilization from the primary tumor, as well as blood vessel formation and increased proliferation of metastatic cells in the lungs in an IL-6/VEGF-A-dependent manner. Our results emphasize the need for long-term follow-up of patients with metastatic disease after treatments that interfere with the tumor microenvironment, such as tumor immunotherapy[58].

2. Examples from current studies from our laboratory:
2.1. Swiss Personalized Oncology

"I have been impressed with the urgency of doing. Knowing is not enough; we must apply. Being willing is not enough; we must do". (Leonardo da Vinci).

The Swiss Personalized Oncology (SPO) driver project, part of the Swiss Personalized Health Network (SPHN), is chaired by myself and Prof. Olivier Michielin (CHUV, Lausanne). SPO is a Switzerland-wide effort that aims at integrating clinical and molecular information from cancer patients, which should ultimately enable more precise diagnoses and thus treatments tailored to individual patients. SPO's main goal is to achieve interoperability of the clinical and laboratory data from cancer patients in Switzerland. We have already made major progress in this challenging but urgently needed endeavor – thanks to the great work of all the SPO centers and their very productive meetings and networking activities, as well as to the tight collaboration with the SOCIBP SPHN driver project led by Prof. Mark Rubin (University of Berne). First, we identified a minimal dataset that specifies the critical data to be harmonized and captured from digital medical records within the routine clinical flow in university hospitals. Furthermore, we composed a digital clinical reporting form to capture these data from non-university cancer clinics (e.g., Swiss Association for Clinical Cancer Research, SAKK). Second, a strong alignment between the SPO Driver project and SAKK was further consolidated, both at the technical and the governance level. Third, we set up the infrastructure of the Swiss Molecular Tumor Board (SMTB), which brings

together experts from the five Swiss university hospitals to discuss complex oncology cases. The originality of the SMTB lies not only in its nationwide format but also in the fact that both clinicians and translational research scientists participate in these meetings. The scope of the SMTB could now be extended from a purely educational board to one delivering clinically relevant input; it will also be expanded to more institutions. Finally, we have assembled retrospective, archived breast cancer and melanoma specimens for broader analysis (e.g., tissue microarrays). Prospectively, we have established and disseminated protocols for live tumor-cell biobanking that have been collated and distributed in coordination with the SAKK. These nationwide efforts have initiated the integration of clinical and molecular information from cancer patients and fostered numerous interactions and fruitful collaborations between clinicians and researchers all over Switzerland.

2.2. Personalized breast cancer treatment: ongoing studies

While the SPO is a nationwide effort, we have founded, together with Prof. Walter Weber (USB), the Basel Breast Consortium (https://baselbc.org), an interdisciplinary organization committed to the development of basic, clinical and translational research projects by supporting interdisciplinary communication and mutual education in Switzerland and neighbouring cities. We have also assembled, a local group of USB colleagues (Surgery, Gynecology, Pathology, Radiology, and Oncology) to make up a breast cancer personalized medicine team that should ultimately improve treatment of patients. Our goal is to collect patient samples and to use multiomics, combined with drug response profiling and computational analysis, in the assessment and modeling of cancer and tumor microenvironment heterogeneity in a longitudinal way. We apply a personalized systems medicine interdisciplinary approach to discover predictive biomarkers and mechanisms of resistance, to identify novel targets, and to rationally design combination therapy. We have already succeeded in establishing many of these approaches. The flagship project focuses on ERα-positive breast cancer and aims to identify mechanisms of resistance to endocrine therapy and CDK4/6 inhibitors using patient material.

ERα-positive breast cancers, which make up the majority of breast cancers (70% of cases), are frequently responsive to endocrine therapy that interferes with estrogen synthesis or signaling. Unfortunately, in 25% of cases, endocrine therapy-resistant metastases develop that initiate an inexorable downhill course. Mechanisms of resistance often culminate in the activation of the Cyclin D1-CDK4/6 complex[59].

Proliferation of cancer cells is often deregulated and sustained chronic proliferation is a fundamental hallmark of cancer[60]. The cell cycle is usually a tightly controlled process and both the serine/threonine cyclin dependent kinases (CDK), their associated regulatory subunits (the cyclins), and their inhibitors (e.g., p16, p21, p27) are important for progression from one phase of the cycle to the next. For example, extracellular signals (e.g., estrogen, growth factors) increase the levels of D-type cyclins during the G1 phase, and the CDK4/6-cyclin D complex triggers transition of cells from early to late G1 phase, progressing through the restriction point gate. The tumor suppressor RB (retinoblastoma related) binds the transcription factor E2F and arrests cells in G1. Both estrogen and growth factors increase D1 expression. The resulting RB phosphorylation and inactivation by the CDK4/6-D complex during the G1 phase allows cells to pass the restriction point. The CDK2-Cyclin E complex induces hyperphosphorylation of RB, thus completing its inactivation and triggering the transition from G1 to S phase. Mechanisms that enhance these transitions are significant in breast cancer initiation and maintenance and include activation of D and E cyclins (e.g., amplification, translocation) and loss of RB or CDK inhibitors[61]. Not surprisingly, inhibition of CDK4/6 has been proposed as a means of treating ERα-positive breast cancers. Several selective CDK4/6 inhibitors (CDK4/6i) have been developed and tested, including Palbociclib/PD0332991, Abemaciclib/LY5219, and Ribociclib/LEE011. Notably, preclinical studies and recent clinical trials (e.g., PALOMA1, 2, MONALEESA2, PALOMA3, MONARCH-1, MONARCH-2, MONARCH-3) have shown the efficacy of combined endocrine therapy and CDK4/6 inhibition in metastatic ERα-positive breast cancers. Ribociclib, Abemaciclib and Palbociclib have been FDA approved in combination with Letrozole for use as first-line therapy in patients with metastatic breast cancer (MBC), and in combination with fulvestrant for patients with MBC who progressed on prior

endocrine therapies[62–70]. While these treatments show high efficacy compared to single endocrine agents, some patients do not respond to such treatment or they develop resistance. The basis of resistance in the clinic remains ill-defined. Possible resistance mutations have been identified, mainly mutations in RB1, PIK3CA and ESR1[71]. Preclinical studies in model systems suggest that loss of RB, overexpression of cyclin E or PDK1, amplification of CDK6, or activation of the D1-CDK2 pathway may account for resistance to CDK4/6i as a single agent[72–75].

To capitalize on the early clinical success of CDK4/6i, it is very important to assess mechanisms of pre-existing and acquired resistance to such inhibitors. We are using different patient-derived *ex vivo* and *in vivo* model systems, including patient-derived organoids (PDOs) and patient-derived xenografts (PDXs), before treatment and after tumor progression. By continuous exposure of PDXs to endocrine therapy and/or CDK4/6i, we are also generating models that are resistant to such treatments (Figure 4). First, we apply a combination of unbiased genomic and proteomic analyses to identify the underlying mechanisms of resistance.

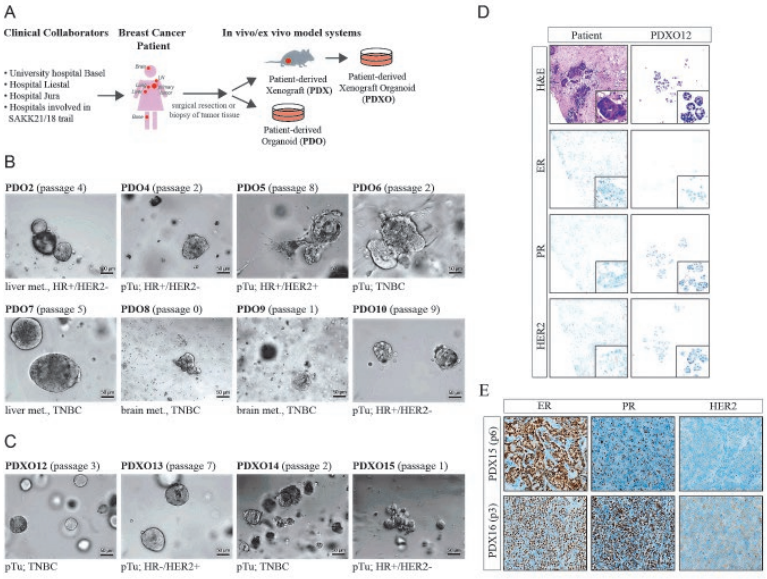

Figure 4. *Patient-derived* ex vivo *and* in vivo *model systems from breast cancer patients.* ***A.*** *Scheme displays our clinical collaborators and patient-derived organoids (PDO), patient-derived xenografts (PDX), and patient-derived xenografts organoids (PDXO) models from different human primary breast tumors (pTu) and metastases (met).* ***B-C.*** *Representative bright field images of established PDO (B) and PDXO (C) cultures.* ***D.*** *Images of sections of a primary tumor and corresponding PDXO models.* ***E.*** *Images of sections of PDXs from ER+ breast tumors. Expression of ER, PR and HER2 was analyzed by IHC.*

Second, we use high-throughput fluorescent microscopy and high-end single-cell imaging in drug sensitivity functional profiling assays (i.e., pharmacoscopy) to discover means to circumvent and overcome CDK4/6i/endocrine therapy resistance, and to develop new mechanism-based personalized therapy for our patients (Figure 5). The future of cancer therapy relies on the diversity of target inhibitors, applied in combination[12,30,56,76,77]. Our studies should lead to the identification of novel personalized combination therapies.

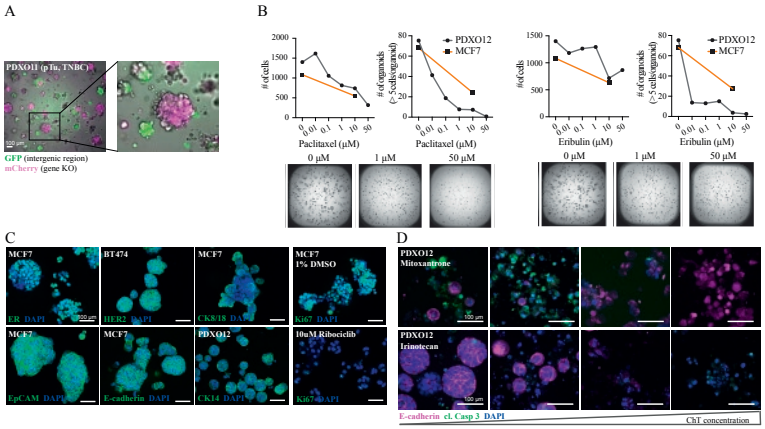

Figure 5. Ex vivo *chemosensitivity functional profiling.* ***A.*** *Bright field image of a PDXO (organoids derived from a TNBC PDX) transduced with vectors targeting an intergenic region (GFP) or a specific gene (mCherry)* (left). *Black box indicates enlarged region* (right). ***B.*** *Image-based drug sensitivity screen: shown are representative whole-well bright field images of organoids treated as indicated and the quantification of single cells and organoids cultured in Matrigel.* ***C-D.*** *Immunofluorescence images of PDXO12 or MCF7 treated and stained as indicated.*

2.3. Effects of mammary tumor heterogeneity on tumor initiation, metastasis, and resistance to therapy: ongoing studies

Transposon insertional mutagenesis: a genetic tool for generating heterogeneity

Transposon insertional mutagenesis is a powerful tool for the discovery of cancer-related genes in mice[78–81]. Indeed, the fact that transposons change their relative position within the genome and alter gene function in cells that express the transposase make these systems ideal for whole-genome screens. The *PiggyBac (PB)* transposon was engineered to be active in mammalian cells[82]: it has higher activity than other transposon systems (e.g., *Sleeping Beauty*)[83]; it moves larger DNA segments[54,56]; it leaves no footprint after transposition; it has a low tendency

for local hopping[78]; it has been used successfully for cancer gene discovery in mice[78–81]. Our lab has been using this genome-wide mutagenesis approach to identify genes and pathways that regulate normal and neoplastic mammary stem cells, the progression to metastasis, and the resistance to therapy. The *PiggyBac* transposon includes two splice acceptors (CβASA, Carp b-actin splice acceptor; En2-SA, Engrailed-2 exon-2 splice acceptor), two poly-A signals (bidirectional SV40 polyadenylation signal, pA), a cytomegalovirus enhancer, a chicken beta-actin promoter (CAG), and a splice donor (Foxf2 exon-1 splice donor, SD). The transposons are mobilized by the *PiggyBac* transposase in a cut-&-paste manner and can be inserted throughout the genome wherever there is a TTAA. This system allows the identification of both oncogenes and tumor suppressor genes, depending on the site of insertion and orientation of the transposon (Figure 6).

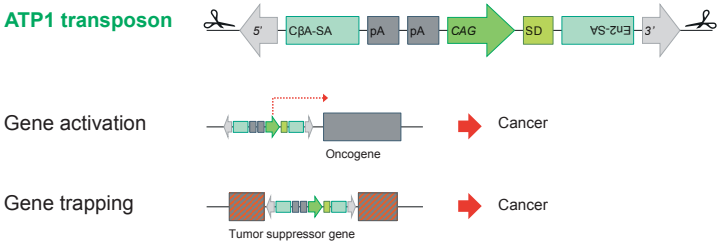

Figure 6. *Design of the transposable element ATP1 and dual mode of action at the integration site*

2.4. Cancer poses a global challenge that requires global efforts: ongoing studies

Together with several colleagues, we have created an international network of labs working on breast biology and cancer (www.enbdc.org) with the goal to foster scientific exchange and collaboration, as well as mutual training and education worldwide.

To develop more fidelitous ex vivo and in vivo models for studying breast cancer, we have teamed up with the labs of Profs. Alana Welm, Brian Welm (Huntsman Cancer Institute, Salt Lake City) and Mike Lewis (Bay-

lor College of Medicine, Houston) to harmonize our respective collections of patient-derived organoids (PDOs) and primary-derived xenografts (PDXs). By combining efforts locally, nationally and internationally, we aim to create synergies that will lead to a better understanding of breast cancer biology and thus more relevant treatments.
Didn't Aristotle say that *"The whole is greater than the sum of its parts"*?

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