# STRESS ADAPTATION AND TUMOR ESCAPE

## Achievements (2020-2025)

Exploration of intra and inter patient response heterogeneity

The variety and complexity of features contributing to cancer ecotype resistance poses a major challenge for the development of predictive markers. A. Basseville implemented mRNA expression data-analysis using machine learning to develop hormone therapy response prediction models out of a cohort of 1,416 breast cancer. Nervous system biomarkers were identified as associated with poor hormone therapy outcome *(P1, F1)*. To improve model prediction performance, we designed an algorithm to better handle high-dimensional omics data which, based on Wasserstein distance, outperforms standard methods for predicting response from transcriptomic data *(P7, F2, collab. Pr F Panloup, LAREMA)*.

On another layer of regulation, PF Cartron (together with F Gautier) aims to characterize how epigenetic and epitranscriptomic programming contribute to resistance. We were pioneers in the identification of m5 C and A modifications of mature miRNAs as critical features of tumor response. These determine temozolomide or PDL1-therapy resistance in glioblastoma and lung cancer *(P2,* Briand et al. Mol Ther Nuc Acids 2020, Briand et al. Epigenomics 2020 , Guyon et al. CDDis 2020*, F3, F4, F5, F6, F11)*. We found using model cell lines and patient blood samples that palbociclib resistance in breast cancer is driven by METTL3-dependent reprogramming of miRNA adenosine methylation, leading to oncogenic protein overexpression and enabling early prediction of resistance through blood biomarkers *(F10, F12; Courant et al., under review)*. Base modified miRNAs have thus a strong potential as patient stratification biomarkers and can be detected in exosomal preparations of liquid biospies. Moreover, even if not necessarily driving resistance, they can generate therapeutic vulnerabilities by modulating expression of actionable actors. The implementation of the Ep'ICO platform, equipped with long read sequencing facilites (*F16)* will allow to exploit this potential.

Protein expression analysis is of particular added value to study stressed biological systems, as it is often uncoupled from RNA expression. F Guillonneau addresses this aspect with the support of ICO, one of the only cancer centers in France to provide an IBISA labeled proteomics platform dedicated to translational research. We set-up with Prot'ICO strategies to obtain and analyze proteome-related data (*P8, P9*, Dumont et al., Lallier et al. BiorXive). After setting up a high-throughput LC-MS system, Prot'ICO enhanced capabilities with a microdissection-capable microscope (LMD 7 Leica) and a picoliter-scale liquid handling system (Cellen-One neoX1). It is now formally possible to isolate, process, and analyze individual cells or small cell populations with minimal loss. Enabling near single-cell proteomic analysis will boost protein expression analysis of heterogenous small-sized cellular populations.

Role of stroma-tumor interactions in stress response

Our apprehension of tumors as multi-cellular ecosystems led F Souazé to explore cancer associated fibroblasts (CAFs), a central coordinator of the cellular microenvironment including nerves and immune cells) in solid tumors. Primary cultures of breast CAFs protect neighboring hormone receptor positive cancer cells from death by enhancing the expression of MCL-1, a BCL-2 family protein which promotes breast cancer cell survival in compensation with BCL-xL *(Louault et al., Oncogene, 2019)*. We established in a co-culture models, using cell lines and Patient-derived Organoids (PDOs) that CAFs reduce sensitivity to chemotherapy even when BCL-xL expression/activity (associated with ex vivo chemotherapy resistance across PDOs) is limited *(P6, F12, F14)*: the stroma adds its protective effects to that of the tumor expressed, chemoresistance marker, BCL-xL.

CAFs are heterogenous themselves and their plasticity renders their own response difficult to decipher. We made a major finding: inhibiting MCL-1, pharmacologically or through gene silencing, mitigates CAF myofibroblastic features, disrupts actomyosin organization and YAP activity, leads to reduced contractility and diminishes pro-invasive effects on cancer cells *(P4, F9, F14)*. Single-cell RNA seq analyses (led by J Derrien) of CAF primary populations after MCL-1 knock down revealed a shift from a wound-healing myfibroblastic state toward an inflammatory, pro-angiogenic profile. Functionally, MCL-1 antagonsim increases VEGF secretion, enhances endothelial cell tubulogenesis, and results in greater peritumoral vascularization in chicken chorioallantoic membrane (CAM) models. This is relevant to stromal response to chemotherapy as the latter lowers MCL-1 levels in CAFs through induction of the endogenous inhibitor NOXA, and activates NF-κB to promote inflammatory and angiogenic programs. This defines MCL-1 as a key molecular switch of CAF phenotype upon treatment *(P8, F9, F14).*

Stress response, inflammation and mitochondrial dynamics

The above results underscore the fine tuning mitochondrial integrity and BCL-2 family proteins might exert on inflammation. S Barillé-Nion investigates this interplay in tumor cells themselves. In an initially assumption free study using breast cancer organotypic cultures (tumor slices), patient derived xenografts and PDOs, we demonstrated that antimitotic chemotehrapy triggers the cGAS–STING pathway to initiate a paracrine TNF and type I interferon dependennt pro-death program relying on NOXA induction *(P1, F12, F13)*. Our study pioneered the rationale for combining STING agonists with BH3 mimetics with an importance of treatments sequence, as fostering mitochondrial outer membrane permeabilization (MOMP) blunts paracriny. We furher evaluated the importance of MOMP intensity in the inflammatory response to treatment by genetic ablation of NOXA in model systems. Strikingly, this does not promote overt survival but changes cell death modes, allowing an inflammatory pyroptotic-like GSDME dependant death to be manisfest. Although the downstream consequences for pro-tumor immunity remain to be characterized, this argues that partial MOMP would favor IL1/IL18 production and sustained inflammation (Dumont et al. BiorXiv: doi: https://doi.org/10.1101/2023.10.06.561231, F12).

## STRESS ADAPTATION AND TUMOR ESCAPE

This interplay between MOMP and inflammatory status, and the fact that the latter influences epithelial phenotypes in breast cancer (Youssef et al. Nature Cancer, 2024), prompted us to consider that some breast cancer cell state transitions or identities may be dependent on BCL-2 protein activity. To evaluate whether specific phenotypes can be targeted by specific BH3 mimetics, J Derrien built a scRNAseq atlas of more than 50 000 tumor cells from a panel of PDOs untreated or counter selected by MCL-1 or BCL-2/BCL-xL antagonists. PDOs display distinct cellular compositions, being predominantly basal or luminal, or mixed. This cutting edge single cell pharmacotranscriptomic approach will highlight complementary roles for MCL-1 and inflammation in maintaining basal characteristics within plastic tumor cell populations *(ms in prep).*

MOMP rates and turnover critical for the inflammatory status are likely affected phenotypes specifically maintained by BCL2 family members by mitochondrial dynamics and inter-organelles contacts. L Lalier specifically studies these and has set up diverse cellular tools to do so, establishing that stress increases contacts between mitochondria, ER and lysosomes, and favor mitochondrial translocation of ceramides or BCL-2 itslef (*Mignard et al. J Lipi Res.2020; Lalier et al. CDDis 2021)*. Unsupervised global proteomic analysis of temozolomide-treated glioma cells coupled to microscopic evaluation and in-situ proximity ligation assays revealed mitochondrial remodeling and quality control mechansims at work during the transition from drug sensitivity to resistance, putting forth the role of transient oxidative stress and of Src–EGFR–TBK1–driven mitophagy in the emergence of resistance (Lalier et al. BiorXiv https://doi.org/10.64898/2025.12.19.695123).

Regulation of stress response by protein interactions

L Maillet studies how BCL-2 proteins regulate mitochondrial integrity through protein interactions at subcellular membranes. The chemoresistance marker BCL-xL in particular exerts its canonical function by binding the BH3 domains of mitochondrial-permeabilizing BAX/BAK and of their upstream regulators BH3-only proteins through a hydrophobic groove. This BH3-binding interface is exposed to the cytosol while BCl-xL C-terminal tail anchors the polypeptide to mitochondrial outer membrane (or the ER). Combining resonance energy transfer assays and molecular dynamics simulations (collab. S Teletchea, US2B), we unraveled that membrane anchoring of BCL-xL selectively advantages binding to membrane-anchored PUMA over soluble BH3 mimetic ligands of the groove (BH3m, first generation of BCL-xL antagonists). This is due to a combined allosteric effect on BH3-in-groove binding of BCL-xL and PUMA TAs. Doubly anchored complexes recruit BAX, which favors their antagonism by BH3mim as a crucial feedback disruptor of the BCL-xL networs. This study supports designing novel, more efficient BCL-xL antagonist startegies based on TA-induced allosteric regulation *(P10, F12, F14)*.

In parallel, PF Cartron investigates the mechanisms at stake in the assembly of protein complexes critical for epigenetic modifications (Cheray et al. Clin Epigenetics 2013, Epigenomics 2014, Theranostics 2016), targeting complexes responsible for transcription-factor–directed DNA methylation (P10, F10).DNMT3A/c-myc, DNMT3L/DNMT3B/p65-NFκB, and DNMT1/ELK1 complexes were identified as regulators of *CDKN2a*, *TRAF1*, and *DGKI* expression, and a molecular rationale for a repression role of the corresponding transcription factors was provided (Hervouet et al. Epigenetics 2009, Pacaud et al. 2014, Biochimie, P10) Importantly, we also uncovered a novel mechanism of RNA-binding protein–directed RNA methylation, whereby a methyltransferase is recruited to specific RNA sequences through interaction with a RNA binding protein, as in the METTL3/RPS3 complex (Courant et al. , under review).

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