CONCLUSION: This is the first comprehensive study describing how tenascin-C promotes tumorigenesis in vivo. Our data suggest that tenascin-C promotes several events leading to metastasis, that will be described in detail. This knowledge is important to combating tenascin-C actions in cancer.

**33** Oral

**Breast tumor environment inhibits human plasmacytoid dendritic cell functions**

V. Sisirak[1], C. Aspord[2], J. Banchereau[3], A.K. Palucka[3], J. Plumas[2], J.Y. Blay[1], C. Caux[1], N. Bendriss-Vermare[1]
*[1]Centre Léon-Bérard INSERM U590, Cytokines & Cancers, Lyon, France; [2] EFS, Recherche et Developpement, Grenoble, France; [3] BIIR, Immunology, Dallas, USA*

Background: A retrospective analysis performed with primary breast carcinomas has reported that the infiltration of plasmacytoid Dendritic Cells (pDC) is associated with an adverse clinical outcome, suggesting that pDCs are involved in breast cancer progression. Indeed the tumor micro-environment may modulate pDC functions for the induction of tumor growth or facilitate the tumor progression by interfering with the immune response.

Material and Methods: To understand the negative influence of the breast tumor environment on human pDC functions, we developed three complementary strategies: 1) an ex vivo phenotypic and functional analysis of breast tumor-infiltrating pDC, 2) an in vitro study of control pDC co-cultured with breast tumor-derived supernatants, and 3) an in vivo model of breast tumors xenograft infiltrated by a pDC cell-line (Gen2.2) in SCID/NOD/b2m-/- mice.

Results: Our first ex vivo studies showed that human breast tumor-infiltrating pDC (Ti-pDC) have an activated phenotype and a lower capacity to produce IFNa in response to Toll Like Receptors (TLR) ligands. We also observed in vitro that breast tumor supernatants specifically inhibited IFNa secretion by activated control pDC. Interestingly as observed with Ti-pDC or normal pDC cultured in presence of breast tumor supernatants, tumor-injected pDC GEN2.2 have mostly an impaired TLR9 responsiveness. Other in vitro studies have also shown that pDC in presence of breast tumor supernatants keep their capacity to induce T-cell proliferation but direct those T-cells to produce high amounts of an immunosuppressive cytokine IL-10. Soluble factors such as TNFa and TGFb which are present in the breast tumor environment seem to be involved in the functional alteration of pDC. Indeed the use of blocking antibodies against TNFa and TGFb restored the production of IFNa by activated pDC in presence of tumor supernatants in vitro. The effect of TNFa and TGFb on the capacity of pDC to induce IL-10-producing Tcells is under investigation.

Conclusions: Our results suggest that the breast tumor microenvironment subverts pDC function in order to maintain tumor tolerance. Further studies are ongoing in our xenograft model in order to validate some new therapeutic approaches that are based on the reversion of the functional inhibition of Ti-pDC to induce an effective antitumor immunity in breast cancer.

**34** Oral

**Molecular subclassification of breast carcinomas based on aCGH, gene expression, IHC and ploidy - relevance for clinical outcome**

H.G. Russnes[1], T. Sørlie[1], A. Krasnitz[2], A. Zetterberg[3], B. Naume[4], E. Borgen[5], J.M. Nesland[5], M. Wigler[2], A.L. Børresen-Dale[1], J. Hicks[2]
*[1]Rikshospitalet-Radiumhospitalet Medical Center, Department of Genetics, Oslo, Norway; [2] Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, USA; [3] Karolinska Institutet, Department of Oncology-Pathology, Stockholm, Sweden; [4] Rikshospitalet-Radiumhospitalet Medical Center, The Cancer Clinic, Oslo, Norway; [5] Rikshospitalet-Radiumhospitalet Medical Center, Dapartment of Pathology, Oslo, Norway*

Identification of well defined molecular subgroups of carcinomas is important for identification of novel therapy targets, for prediction of response and for improvement of prognostication. A molecular taxonomy for breast cancer based on expression profiling identified five subgroups: luminal A, luminal B, basal-like, ERBB2+, and normal-like (Perou et al. Nature 2000, Sørlie et al. PNAS 2001). Based on genomic alterations Hicks et al. (Genome Res 2006) identified three different patterns of alterations, “simplex”, ”complex” and “firestorm”. The aim of this study was to explore both genomic, gene expression and protein data from early stage breast carcinomas to develop a combined and robust classifier that distinguishes between distinct biological subgroups with clinical relevance.

Tumor tissue from 137 early stage breast cancer patients was analyzed for genomic alterations by high-resolution aCGH, HER2 amplification by FISH, TP53 mutation by sequencing, expression subclasses by DNA microarrays, and ploidy and protein expression using tissue micro arrays (TMA). We designed a CGH classifier based on known genomic alterations characteristic of the intrinsic subgroups, and applied a mathematical algorithm on the aCGH data that defined loss and/or gains of whole arms in addition to more complex alterations (“firestorms”).

Based on the developed CGH classifier, a luminal (48%), non-luminal (20%), a mixed (11%) and an unclassified (20%) group was identified. The luminal subgroup was dominated by loss or gains of whole chromosome arms. More than half were diploid, the rest aneuploid. Most tumors were ER+ (82%) and only 7% HER2+. 62% of this group was lumA by gene expression, the remaining lumB, ERBB2+ or normal-like. The non-luminal subgroup showed more complex genomic alterations, 50% were basal-like and 44% were either luminal B or ERBB2+ by gene expression. 81% had TP53 mutations. This group could further be stratified by HER2 status; the HER2+ were aneuploid and either ERBB2+ or lumB by gene expression; the HER2- were CK5/6+ and/or 17+ by IHC, either diploid or aneuploid, and basal-like by gene expression. Samples in the mixed and the unclassified subgroups were mostly aneuploid, and all expression subclasses were represented.

Our data from combined molecular profiling identify relevant clinical subgroups with different outcome. These results have to be validated in a larger cohort.

**35** Oral

**Activation of alternative HER receptors mediates resistance to EGFR tyrosine kinase inhibitors in breast cancer cells**

A. Kong[1], V. Calleja[1], P. Leboucher[2], A. Harris[3], P. Parker[4], B. Larijani[1]
*[1]Cancer Research UK, Cell Biophysics Lab, London, United Kingdom; [2] College de France, 11 Place Marcelin Berthelot, Paris, France; [3] Weatherall Institute of Molecular Medicine, Cancer Research UK Molecular Oncology, Oxford, United Kingdom; [4] Cancer Research UK, Protein Phosphorylation Lab, London, United Kingdom*

The response rate to EGFR inhibitors may be poor and unpredictable in cancer patients with EGFR expression itself being an inadequate response indicator. There is limited understanding of the mechanisms underlying this resistance. Here we have provided a molecular mechanism of alternative HER receptor activation (ErbB receptor family members) in mediating resistance to EGFR TKIs in breast cancer cells. Using both Förster Resonance Energy Transfer (FRET) which monitors in situ HER receptor phosphorylation as well as classical biochemical analysis, we have shown that the specific tyrosine kinase inhibitors (TKIs) of EGFR (HER1), AG1478 and Iressa (Gefitinib) decreased EGFR and HER3 phosphorylation through the inhibition of EGFR/HER3 dimerization. Consequent to this, we demonstrate that cleavage of HER4 and dimerization of HER4/HER2 occur together with reactivation of HER3 via HER2/HER3, leading to persistent HER2 phosphorylation in the now resistant, surviving cells. These drug treatment–induced processes were found to be mediated by the release of ligands including heregulin and betacellulin that activate HER3 and HER4 via HER2. Whereas an anti-betacellulin antibody in combination with Iressa increased the anti-proliferative effect in resistant cells, ligands such as heregulin and betacellulin rendered sensitive SKBR3 cells resistant to Iressa. These results demonstrate the role of drug-induced autocrine events leading to the activation of alternative HER receptors in mediating resistance to EGFR tyrosine kinase inhibitors (TKIs) in breast cancer cells, and hence specify treatment opportunities to overcome resistance in patients.

**36** Oral

**Large scale comparative proteomic study of accessible vascular proteins in mouse liver metastases and normal liver**

B. Borgia[1], C. Rösli[1], T. Fugmann[1], D. Neri[1], R. Giavazzi[2]
*[1]ETH Zurich Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, Zurich, Switzerland; [2] “Mario Negri” Institute for Pharmacologic Research, Department of Oncoloy, Milan, Italy*

INTRODUCTION: The aim of our study is the identification of tumour associated antigens (TAA) localized at newly formed blood vessels or in the surrounding stroma as a tool for the development of novel antibody-based therapy with special focus on the metastatic process.

METHODS: Three murine cancer cell lines metastasizing to the liver (M5076, Colon38 and SL4) were injected into C57BL/6 mice and tested for their metastatic potential. Tumour-bearing and healthy mice were subjected to terminal perfusion with a reactive ester derivative of biotin (Sulfo-NHS-LC-Biotin) in order to chemically modify accessible membrane and extracellular matrix proteins from the bloodstream. Biotin labelled proteins are purified on streptavidin resin, trypsinized on resin and subsequently analysed by RP-nano-HPLC and MALDI-TOF/TOF procedures. Peptides are identified by the Mascot software and relatively quantified by 2D-peptide maps using the DeepQanTR software.

RESULTS: Three different syngenic mouse models were set up in order to reproduce and study the complex hepatic metastasis process. Namely, M5O76 (mouse reticulum sarcoma), Colon38 (mouse colon carcinoma) and its highly metastatic variant SL4. Mice were then subjected to the in vivo biotinyation technique and biotinylated organs were excised for further analysis. Successful biotinylation of vascular structures was assessed by histochemical analysis using streptavidin-alkaline phosphatase complex.