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EuCC Symposium 2008

Friday, May 23, 2008,
Dorint Hotel, Basel, Schönaustrasse 10

9:00 Introductory Remarks

9:10 **M. Roth, Strasbourg**
The transmembrane domain of NRP1 is a new target to develop anti-angiogenic strategies

9:35 **Jean-Louis Boulay, Basel**
Loss of *NOTCH2* Positively Predicts Survival in Subgroups of Human Glial Brain Tumor

10:00 **Gertraud Orend, Strasbourg**
Tumorigenesis-promoting events and signaling by Tenascin-C

10:25 Coffee Break

11:00 **Christine Dierks, Freiburg**
Expansion of Bcr-Abl positive leukemic stem cells is dependent on hedgehog pathway activation

11:25 **Ivo Jivkov, Strasbourg**
Overexpression of the laminin α1 chain promotes tumor angiogenesis and invasion

11:50 **Don Benjamin, Basel**
Metformin and hyperthermia inhibits proliferation in a TOR-pathway addicted tumour model

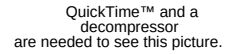
12:15 **A. C. Jung, Strasbourg**
A genomic and transcriptional analysis of HPV-infected oropharyngeal head and neck squamous cell carcinoma

12:40 Lunch Break

14:30 **Björn Hackanson, Freiburg**
Dual epigenetic control of CCAAT/enhancer binding protein α (C/EBPα) expression in acute myeloid leukemia

14:55 **Dominique Guenot, Strasbourg**
Search of molecular markers for human colon tumor progression

15:20 **Michal Rajski, Basel**
IGF-I-induced genes in tumor and stroma cells subdivide human breast cancer in four subgroups with significantly different prognosis



15:45 **Andreas Wicki, Basel**
Synergism of glucagon-like-peptide-1 (GLP-1) receptor targeted therapy with anti-angiogenic compounds in a mouse model of neuroendocrine tumors

16:10 Coffee Break

16:40 **Robert Zeiser, Freiburg**
Preemptive HMG-CoA reductase inhibition provides graft-versus-host disease protection by Th-2 polarization while sparing graft-versus-leukemia activity

17:05 **Nermin Raafat, Basel**
Modulation of immunogenicity of recombinant vaccinia virus as a cancer vaccine

17:30 **Silvia Rathmann, Freiburg**
Expansion of natural killer cells lacking broadly reactive inhibitory receptors post allogeneic hematopoietic cell transplantation

17:55 Meeting Adjourn

The transmembrane domain of NRP1 is a new target to develop antiangiogenic strategies

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We recently found that NRP1 transmembrane domain is important for receptor dimerization and related biological function of this semaphorin and VEGF receptor. FRET analysis and a bacterial dimerization assay (ToxLuc) demonstrated the high dimerization capacity of this domain. Moreover, a synthetic peptide mimicking this segment prevented Sema3A signalling in different cellular assays (inhibitory and growth promoting effects) and also decreased NRP1/Sema3A binding. This peptide partially disrupted receptor complex formation as seen in sucrose gradients analysis. These effects were related to the peptide capacity to interfere with NRP1 dimerization and thereby with the formation of a highly ordered oligomeric complex. Here, we provide evidence for a therapeutic use of this peptide in the context of brain tumour development. Both in vitro and in vivo data demonstrate that tumour growth is strongly reduced in the presence of the peptide. This antitumor effect can be explained by an antiangiogenic activity of the peptide. Overall, our results demonstrate the importance of NRP1 transmembrane domain as a new target to develop innovative antiangiogenic strategies.

Loss of *NOTCH2* Positively Predicts Survival in Subgroups of Human Glial Brain Tumors

Jean-Louis Boulay[1], André R. Miserez[2], Christian Zweifel[1,3], Balasubramanian Sivasakaran[1], Veronika Kana[1], Anthony Ghaffari[1,3], Cordelia Luyken[4], Michael Sabel[4], Abdessamad Zerrouqi[5], Morten Wasner[3], Erwin Van Meir[5], Markus Tolnay[6], Guido Reifenberger[4] & Adrian Merlo[1,3]

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The structural complexity of chromosome 1p centromeric region has been an obstacle for fine mapping of tumor suppressor genes in this area. Loss of heterozygosity (LOH) on chromosome 1p is associated with the longer survival of oligodendroglioma (OD) patients. To test the clinical relevance of 1p loss in glioblastomas (GBM) patients and identifiy the underlying tumor suppressor locus, we constructed a somatic deletion map on chromosome 1p in 26 OG and 118 GBM. Deletion hotspots at 4 microsatellite markers located at 1p36.3, 1p36.1, 1p22 and 1p11 defined 10 distinct haplotypes that were related to patient survival. We found that loss of 1p centromeric marker D1S2696 within *NOTCH2* intron 12 was associated with favorable prognosis in OD (*P*=0.0007) as well as in GBM (*P*=0.0175), while 19q loss, concomitant with 1p LOH in OD, had no influence on GBM survival (*P*=0.918). Assessment of the intra-chromosomal ratio between *NOTCH2* and its 1q21 pericentric duplication *N2N* (N2/N2N-test) allowed delineation of a consistent centromeric breakpoint in OD that also contained a minimally lost area in GBM. OD and GBM showed distinct deletion patterns that converged to the *NOTCH2* gene in both glioma subtypes. Moreover, the N2/N2N-test disclosed homozygous deletions of NOTCH2 in primary OD. The N2/N2N test distinguished OD from GBM with a specificity of 100% and a sensitivity of 97%. Combined assessment of *NOTCH2* genetic markers D1S2696 and N2/N2N predicted 24-month survival with an accuracy (0.925) that is equivalent to histological classification combined with the D1S2696 status (0.954) and higher than current genetic evaluation by 1p/19q LOH (0.762). Our data propose *NOTCH2* as a powerful new molecular test to detect prognostically favorable gliomas.

TUMORIGENESIS-PROMOTING EVENTS AND SIGNALING BY TENASCIN-C

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BACKGROUND: The ECM component tenascin-C is highly expressed in most solid tumors. Its high expression correlates with a bad survival prognosis in patients with several cancers. Results from cell culture experiments support a role of tenascin-C in enhancing tumor cell proliferation, promoting angiogenesis, invasion and metastasis. We showed that tenascin-C induces cell rounding by two mechanisms. Tenascin-C counteracts the tumor cell proliferation-suppressing effect of fibronectin by blocking the integrin alpha 5 beta ı/syndecan-4 complex. This caused cell rounding (Orend et al., 2003, Oncogene 22, 3917) and stimulated tumor cell proliferation (Huang et al., 2001, Cancer Res. 61, 8586) by activation of oncogenic Wnt and MAPkinase signaling (Ruiz et al., 2004, Cancer Res. 64, 7377). Tenascin-C also stimulated endothelin receptor type A (EDNRA) expression, and signaling through EDNRA maintained cell rounding (Lange et al., 2007, Cancer Res. 67, 6163). By using knockdown and overexpression studies, we identified paxillin, RhoA and TM1 as critical targets of cell rounding by tenascin-C downstream of syndecan-4 and EDNRA (Lange et al., 2007, Cancer Res. 67, 6163).

MATERIAL & METHODS: To determine a potential tumorigenesis-promoting effect of tenascin-C *in vivo*, we generated transgenic mice that ectopically express human tenascin-C in the pancreatic islets. Tenascin-C-transgenic mice, that are apparently healthy and fertile, exhibit normal development of the pancreas, but showed enhanced angiogenesis in the pancreatic islets. Next, we crossed RipTNC mice with tumor-prone RipTag2 (RT2) mice, that develop insulinomas due to ectopic expression of the SV40T-antigen and compared tumorigenesis in RT2/TNC and RT2 mice.

RESULTS: Double transgenic RT2/TNC mice experience more frequent and earlier death incidences than RT2 mice. RT2/TNC mice exhibit several signs of enhanced tumor progression such as the appearance of local and distant metastasis.

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. This knowledge is important to combating tenascin-C actions in cancer.

Expansion of Bcr-Abl positive leukemic stem cells is dependent on hedgehog pathway activation

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Chronic Myeloid Leukemia (CML) is characterized by the expansion of a leukemic stem cell clone carrying a Philadelphia translocation, which outgrows the non-malignant haematopoiesis. The mechanisms how the Bcr-Abl positive leukemic stem cell pool expands during chronic phase of disease is not yet understood. Current targeted therapies against the Abl kinase with STI571 (Gleevec) or AMN107 (Nilotinib) fail to eradicate the complete leukemic stem cell pool. Therefore patients are forced to a live long therapy and resistant clones can evolve and cause relapse of disease. These clinical findings indicate that new therapeutic targets have to be identified which are specific for the Bcr-Abl positive leukemic stem cells.
By comparing the gene expression profile of murine Bcr-Abl positive and negative hematopoietic stem cells, we identified Gli1 as one gene which is specifically upregulated in Bcr-Abl positive leukemic stem cells (Lin-Kit+Sca+). These results could be reconfirmed by using taqman PCR and IHC staining for Gli1 in the bone marrow of CML patients. In regular hematopoiesis Gli1 expression levels and its activity as transcription factor are regulated by the hedgehog signaling pathway upon ligand stimulation of the receptor Ptch with Ihh or Shh. In contrast to regular haematopoiesis we observed that BCR-ABL intrinsically activates the hedgehog signaling pathway by upregulation of the receptor Smo in murine and human Bcr-Abl positive bone marrow cells. Constitutive hedgehog pathway activaton in murine bone marrow cells by retroviral overexpression of Smo or loss of Ptch ($Ptch^{+/-}$ bone marrow) induced expansion of short term repopulating HSCs *in vitro* and *in vivo* and induced outgrowth of regular hematopoiesis. Therefore intrinsic activation of hedgehog signaling by upregulation of Smo might be an important mechanism for the expansion of the leukemic stem cell pool in CML.
Pharmacological inhibition of Smo activity in Bcr-Abl positive bone marrow cultures by Cyclopamin induced apoptosis of Bcr-Abl positive bone marrow cells to a much greater extent than in the Bcr-Abl negative population. Cyclopamin treatment also reduced the number of murine Bcr-Abl positive leukemic stem cells and the number of colony forming Bcr-Abl positive cells *in vitro* and *in vivo*. Furthermore treatment of bone marrow cultures from CML patients with Cyclopamin reduced the number of long-term culture initiating cells (LTC-ICs) by more than 70% compared to only 10% reduction in LTC-IC cultures from regular bone marrow.
By using fetal HSCs from $Smo^{-/-}$ mice we further established the role of hedgehog signaling in regular versus malignant hematopoiesis on a genetic level. Transplantation of $Smo^{-/-}$ fetal HSCs into irradiated recipients resulted in normal regeneration of the bone marrow besides complete loss of CD8+ T-cells and reduced expansion capacity of the short term repopulating HSCs. The homing, expansion and maintenance of long-term repopulating HSCs was not affected by the loss of Smo. In contrast loss of Smo dramatically delayed the onset of Bcr-Abl positive leukemias in mice (2 weeks with regular bone marrow, 3 months with $Smo^{-/-}$ bone marrow) and reduced the incidence of the disease from 100% to 60%. Retransplantion of leukemic cells isolated from the primary recipients into secondary recipients was completely abolished in Bcr-Abl pos. bone marrow cells lacking Smo, indicating a complete loss of the leukemic stem cell pool upon loss of Smo.
To further establish the role of hedgehog signaling in already developed disease, we induced a CML-like syndrom in mice. Combined treatment of leukemic mice with AMN107 (Abl inhibitor) and Cyclopamine (Smo inhibitor) lead to a reduction of Bcr-Abl positive self-renewing cells *in vivo* and enhanced the time to relapse more than 3-fold compared to mice treated with AMN107 alone.
Thus we conclude that the expansion of Bcr-Abl positive leukemic stem cells is dependent on intact hedgehog signaling. Therefore Hh pathway inhibition alone or in combination with Abl inhibitors could serve as an effective therapeutic strategy to reduce the malignant stem cell pool in BCR-ABL positive leukemias.

Overexpression of the laminin α1 chain promotes tumor angiogenesis and invasion

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Each year more than 100 patients are diagnosed with colorectal cancer in France, which develop into metastasizing carcinoma causing a death toll of more than 16.000 every year. The transition from a carcinoma *in situ* to invasive carcinoma and metastasis requires cancer cell trespassing of the basement membrane (BM) underlying the colonic epithelium and surrounding blood and lymphatic vessels. Major components of BM are laminins. Laminins are large heterotrimeric glycoproteins composed of an α, β and γ chain. The laminin α1 (LNα1) chain in laminin 111 is expressed early during embryogenesis and is essential, since its genetic ablation caused early stage embryonic lethality (Alpy et al. 2005). A high expression of laminin-111 in colorectal carcinoma suggests a role of this laminin in cancer progression. Indeed, laminin-111 strongly enhanced tumor angiogenesis and accelerated tumor growth of xenografted colonic tumor cells overexpressing the LNα1 chain in nude mice (De Arcangelis et al. 2001).
To investigate a potential tumorigenesis-promoting effect of the LNα1 in more detail, we generated transgenic mice that overexpress the LNα1 chain in the intestine (duodenum and colon) under control of the villin promoter. Upon crossing with mice exhibiting a mutated APC allele (APC $^{+/1638N}$; Fodde et al. 1997), we observed that double transgenic LNα1/APC $^{+/1638N}$ mice developed more tumors than APC $^{+/1638N}$ mice. Upon treatment with the carcinogen azoxymethan (AOM) transgenic LNα1 mice developed colonic tumors faster than AOM treated wildtype littermates. Moreover, AOM-induced tumors from LNα1 mice were highly invasive.
Together, our results obtained from three different cancer models demonstrate that uncontrolled ectopic expression of LNα1 stimulates tumor growth and promotes tumor progression.

Metformin and hyperthermia inhibits proliferation in a TOR-pathway addicted tumour model

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IL-3 dependent PB3c murine mastocytes lose the requirement for IL-3 upon oncogenic transformation. We generated IL-3 independent loss-of-function mutants by frame-shift mutagenesis that were sensitive to the mTOR inhibitor rapamycin which triggered apoptosis leading to cell death within 48 hours. Characterisation of the mutants revealed constitutive mTOR activation as confirmed by phospho-readouts either through loss of PTEN (3 mutants), TSC2 (1 mutant) and unidentified genetic lesions (3 mutants).

A microarray analysis for genes showing altered expression in the transition to IL-3 independence or back (by back-fusion to precursor cells), as well as under rapamycin treatment, revealed a single candidate: fructose-1,6-bisphosphatase 2 (FBP2), a key enzyme in gluconeogenesis. Quantitative real-time PCR confirmed FBP2 over-expression in the PTEN-/- mutants relative to precursor cells and that the expression was rapamycin sensitive. Reintroduction of PTEN downregulated FBP2 expression while PTEN downregulation in precursor cells triggered FBP2 expression. When challenged with metformin, a widely used anti-diabetic drug with potent anti-gluconeogenic properties, the mutants were effectively killed with the exception of the TSC2-/- mutant, the sole non-FBP2 expressing mutant. The killing effect of metformin synergised strongly with rapamycin at low doses that may be clinically deliverable.

A related aspect of FBP2 expression is that the simultaneous operation of both gluconeogenic and glycolytic pathways establishes a futile substrate cycle that consumes ATP which is released as heat. It has long been known that cancer cells have higher temperatures due to increased glycolysis but are nevertheless paradoxically more sensitive to temperature elevation (>43.5°), a feature that has been exploited in some alternative treatments. We tested parallel cultures of the various cell-lines at 37° and 44.5° and measured cell viability after 4hrs. The FBP2 overexpressing cells were heat-sensitive (>70% cell death) compared to the non-expressing controls (<20% cell death). Heat sensitivity in FBP2 expressing cells was also strongly potentiated by pretreatment with metformin or rapamycin. As corroboration, re-expression of PTEN in a -/- mutant, which led to a decline in FBP2 expression, also showed a concomitant decrease in heat sensitivity. These results point to an interface between metabolism and oncogenic transformation and FBP2 appears to be a useful marker to indicate cells responding to rapamycin, metformin, hyperthermia or their combined application.

A GENOMIC AND TRANSCRIPTIONAL ANALYSIS OF HPV-INFECTED OROPHARYNGEAL HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinoma (HNSCC) are the fourth more frequent malignancies in France and represent a significant cause of death by cancer in men. Depending on the stage of the lesions, HNSCC are treated with a sequential combination of surgery with curative intend and radiation or chemoradiation therapy. They are often diagnosed at an advanced stage, and have therefore a high propensity for local relapse and lymph node invasion. Thus, despite recent progresses in terms of treatment, prognosis for HNSCC remains poor, with an overall survival at five years lower than 30%.

The major risk factor for the onset and development of HNSCC is the synergistic action of tobacco smoking and alcohol drinking. However, accumulating evidences suggest that the infection by the human papillomavirus (HPV) would be involved in about 25% of cancers of the head and neck. Interestingly, patients bearing HPV-infected lesions seem to constitute a distinct subpopulation: they are often younger (less than 50 years old) and they more often have no history of smoking and drinking. The HPV-infected tumours are more often found in the oropharyngeal region (particularly the tonsils and the base of the tongue) and display non/poorly differentiated and basaloid features. Surprisingly, despite these severe characteristics, HPV-positivity seems to be a better prognosis factor, as it is associated with an increased disease-free and overall survival as compared to HPV-negative tumours. Indeed, HPV-dependent HNSCC are thought to be more radio- and chemoradiosensitive.

These differences in terms of clinical and biological features rely on the fact that HPV-negative and HPV-positive tumours display distinct genetic profiles, mainly due to the specific oncogenic action of the virus. For instance, the E6 viral oncoprotein triggers the degradation of the *TP53* tumour suppressor gene product, whereas *TP53* is found to be mutated in the majority of HPV-negative lesions. Basal levels of non-degraded p53 protein would be sufficient to induce infected cell death upon ionising radiations treatment, and would, at least partially, explain the enhanced local control of radiotherapies on the development of the disease, leading to a longer survival. However, the radiosensitivity of HVP-positive HNSCC remains poorly understood.

In order to gain insight in the molecular basis of the biological and clinical differences observed between HPV-positive and HPV-negative HNSCC, we have performed a genome-wide analysis comparing the genetic and transcriptional profiles of infected and non-infected oropharyngeal tumous samples. We found out 645 probes sets to be significantly misregulated in HPV-positive tumours. These lesions also display a chromosomal loss of the 16q region. Interestingly, a cluster of genes located in 16q shows a down-regulated expression. Among them the loci encoding the Amyloid Precursor Protein Binding Protein 1 (APPBP1) might be an interesting candidate gene with respect to the increased radiosensitivity of HPV-positive HNSSC.

Dual epigenetic control of CCAAT/enhancer binding protein α (C/EBPα) expression in acute myeloid leukemia

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Functional loss of C/EBPα, a master regulatory transcription factor in the hematopoietic system, can result in a differentiation block in granulopoiesis and thus contribute to leukemic transformation. Here we demonstrate the impact of epigenetic aberrations in regulating C/EBPα expression in acute myeloid leukemia (AML). Comprehensive DNA methylation analyses of C/EBPα's CpG island identified a densely methylated upstream promoter region in 51% of AML patients. Aberrant DNA methylation was strongly associated with two generally prognostically favourable cytogenetic subgroups: inv(16) and t(15;17). Surprisingly, while epigenetic treatment increased C/EBPα mRNA levels, C/EBPα protein became silenced. Using a computational microRNA prediction approach and functional studies, we show that C/EBPα mRNA is a target for microRNA-124a. This microRNA is frequently silenced by epigenetic mechanisms in AML, becomes up-regulated following epigenetic treatment and targets the C/EBPα 3'untranslated region. In this way, C/EBPα protein expression is reduced in a posttranscriptional manner. Our results indicate that epigenetic alterations of C/EBPα are a frequent event in AML and that epigenetic treatment can result in down-regulation of a key hematopoietic transcription factor.

Search of molecular markers for human colon tumor progression

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Colon cancers are classified in tumors of microsatellite instability phenotype (MIN) which results from mismatch repair gene alterations and in tumors of chromosomal instability phenotype (CIN) which present mostly aneuploidy. The genetic alterations found in CIN tumors are greatly heterogeneous suggesting that the signalling pathways required for colon carcinogenesis could be more complex than currently proposed. This could account for the development of tumors resistant to chemotherapy and for the difficulty to find efficient anticancer agents. Also, the heterogeneity of the cohorts analyzed could explain why despite numerous published studies, no molecular markers for tumor progression and metastatic invasion or for sensitivity to chemotherapy are available. To define genomic profiles of aberrations linked to the tumour progression, allelotyping completed with a pan-genomic CGH array (aCGH) have been performed on CIN colon tumors. Analysing chromosome 20q status underlined i) tumors with a full gain of the 20q resulting from aneusomy, ii) tumors with disomic 20q and iii) tumors with a full gain of 20q resulting from tetrasomy. The 20q gain was significantly associated to TP53 allelic imbalance and the disomic 20q was found in tumors with a very low frequency of aberrations. Among other aberrations, a microdeletion in 1p36.12 region has been observed for 44% of the tumors of all clinical stages. This genomic region includes several genes and among them, E2F2. E2F family members are key regulators of the cell cycle and apoptosis and the promotion or suppression of proliferation/apoptosis is depending on the cell context. Real-time PCR showed that deletion of the E2F2-targeting probes correlated with a decreased gene copy number and sequencing of the 8 exons did not evidenced gene mutation but a polymorphism in exon 4 was found. At the level of the gene expression, E2F2 mRNA was decreased in distal tumors but increased in proximal tumors. As the clinical data are available for part of the studied tumors, the potential prognostic value of the E2F2 deletion, associated or not to other(s) alteration(s), will be evaluated. The functional impact of the deletion of E2F2 remains to be demonstrated in colon cancer by functional approaches like gain- and loss-of-function.

IGF – I induced genes in tumor and stroma cells subdivide human breast cancer in four subgroups with significantly different prognosis

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Introduction
Insulin-like Growth Factor I (IGF – I) signaling has a key influence on normal breast development, but also on breast cancer initiation and progression. High levels of circulating IGF – I are known to be a risk factor for breast cancer. IGF - I is mainly synthesized by stromal cells, influencing both: the tumor cells as well as the stromal cells. The specific and eventually differential influence of IGF – I on global gene expression of tumor and stroma cells is not yet well characterized. We hypothesized that specific gene expression signatures in stromal and cancer cells induced by IGF – I might be of clinical relevance.

Methods
To characterize the specific and differential gene expression changes due to IGF – I stimulation we set up an *ex vivo* culture model with human CCL - 171 fibroblast and MCF – 7 breast cancer cells and determined IGF – I induced gene expression changes with cDNA microarrays. Obtained gene expression signatures were evaluated with data from two publicly available datasets: 85 locally advanced breast carcinomas from Norway/Stanford and 295 early stage breast cancers (stage 1 and 2) from the Netherlands Cancer Institute (NKI).

Results
Upon IGF – I stimulation CCL – 171 cells significantly changed their gene expression profile with sets of up and down regulated genes. The set of induced genes consisted of many different genes including M-phase cell cycle as well as proliferation associated genes. To check the *in vivo* effects, we verified our data with Norway/Stanford and NKI data. In both datasets, the expression of the CCL – 171, IGF – I induced genes was remarkably coherent, providing a basis for segregation of the tumors into two groups. In a univariate analysis, tumors with high expression levels of IGF – I induced genes had a significantly shorter disease specific survival time ($p=0.0294$ for the Norway/Stanford dataset and $p=2.07e-09$ for the NKI dataset) than tumors with low expression levels. Furthermore in NKI dataset we found a clear segregation of the tumors within the two main groups into four subgroups. These four tumor subgroups had significantly different overall survival times ($p=1.51e-08$). The four subgroups are determined by the two strong clusters of genes. These two gene clusters can be distinguished by the dominant expression of genes in the tumor cells in one cluster and of stromal genes in the other cluster. Interestingly, deregulation of the stromal gene cluster led to a worse prognosis than deregulation of tumor gene cluster.

Conclusion
Genes induced by IGF – I *in vitro* are strongly prognostic in human breast cancer. These genes can be separated *in vivo* into two clusters one of which is expressed in the tumor cells and the other in the stromal cells. Differential expression of the stromal cluster seems to be prognosticaly dominant over the tumor gene cluster, implicating an important role in cancer progression and as potential targets of stroma directed antineoplastic therapies.

Synergism of glucagon-like-peptide-1 (GLP-1) receptor targeted therapy with anti-angiogenic compounds in a mouse model of neuroendocrine tumors

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Purpose: Neuroendocrine tumors are well vascularized and usually express specific cell surface markers, such as somatostatin receptors. Using a transgenic mouse model of human insulinoma, we have investigated the potential benefit of a combination of anti-angiogenic treatment with targeted internal radiotherapy. To this end, [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-Exendin-4, a radiopeptide that selectively binds to the GLP-1R overexpressed on insulinoma cells, was co-administered with PTK787, a tyrosine kinase inhibitor of the VEGFR-2 pathway.
Experimental design: For biodistribution, Rip1Tag2 mice were pre-treated with PTK787 for 0, 3, 5 or 7 days. Then, [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-Exendin-4 was injected and the biodistribution assessed after 4 hours. For therapy, mice were injected with 1.1 MBq [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-Exendin-4 and treated with PTK787 orally for another 7 days. Thereafter, tumor volume, tumor cell apoptosis, proliferation and microvessel density were quantified.
Results: Pre-treatment of mice with PTK787 led to a marked reduction in the tumor uptake of [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-Exendin-4, making a simultaneous start of the oral PTK787 treatment with the injection of the radiopeptide the most efficient strategy. Combination of [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-Exendin-4 i.v. and PTK787 p.o. led to a reduction of the tumor volume by 97% without concomitant organ damage.
Conclusions: In this pre-clinical study, combination of targeted internal radiotherapy with an anti-angiogenic compound inhibiting the VEGFR-2 axis had an additive effect on therapy efficiency. The combination of 1.1 MBq of [Lys^{40}(Ahx-DTPA-^{111}In)NH_2]-Exendin-4 with PTK787 had the same effect on a neuroendocrine tumor as the injection of 28 MBq of the radiopeptide alone, but without side-effects such as radiation damage of the kidneys.

Preemptive HMG-CoA reductase inhibition provides graft-versus-host disease protection by Th-2 polarization while sparing graft-versus-leukemia activity

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Allogeneic hematopoietic cell transplantation is a potentially curative option for patients with hematological malignancies. However, the overall success of this approach is limited by the occurrence of acute graft-versus-host disease (aGvHD). We investigated whether atorvastatin (AT) was capable of protecting animals from aGvHD across major-MHC mismatch barriers. AT treatment of the donor induced a Th-2 cytokine profile in the adoptively transferred T cells and reduced their in vivo expansion, which translated into significantly reduced aGvHD lethality. Host treatment down-regulated costimulatory molecules and MHC class II expression on recipient APCs and enhanced the protective statin effect. Importantly we found tha Graft-versus-tumor (GvT) activity was maintained in two different murine tumor models. The AT effect was partially reversed in STAT6$^{-/-}$ donors and abrogated by L-mevalonate, indicating the relevance of STAT6 signaling and the L-mevalonate pathway for AT mediated aGvHD protection. AT reduced prenylation levels of GTPases, abolished T-bet expression and increased c-MAF and GATA-3 protein in vivo. Thus, AT has significant protective impact on aGvHD lethality by Th-2 polarization and inhibition of an uncontrolled Th-1 response while maintaining GvT activity.

Modulation of immunogenicity of recombinant vaccinia virus as a cancer vaccine

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Background
Although many reports have highlighted the potential of poxviral vectors as recombinant vaccines, their immunogenicity can also be a major drawback. Indeed, immunodominant vector-specific CTL response could limit the effectiveness of recombinant poxviruses especially in cancer immunotherapeutic strategies which often require multiple rounds of vaccine stimulations. To balance this effect, powerful heterologous prime-boost strategies or immuno-modulation of vector specific responses are required.

Objective
We aim at decreasing CTL response against Vaccinia Virus by diminishing the viral epitope MHC class-I restricted presentation from infected cells without affecting the presentation of recombinant TAA epitopes encoded by minigenes or MHC class-II presentation of viral entities. This approach should simultaneously decrease epitope competition and the CD8 anti-vector responses.

Hypothesis - Design
ICP47 protein (encoded by US12 gene from HSV-I) has been shown to interact with Transporter of Antigen Processing (TAP) protein thereby inhibiting peptide transport to the Endoplasmic Reticulum. This peptide blockade prevents MHC-I loading and surface presentation.

We anticipated that in antigen presenting cell infected with recombinant vaccinia virus expressing US12 gene, the generation of epitopes derived from viral proteins should be blocked. In contrast, recombinant ER-targeted vaccine epitopes should not be affected and their overall immunogenicity may be increased.

Methods
Herpesvirus US12 gene was introduced into Vaccinia virus wild type as well as the rVV expressing the ER-Mart$_{27-35}$, a melanoma associated HLA-A2 restricted epitope. Effect on MHC-class I and other surface molecules from infected cells (using non replicating virus) was characterized by antibody staining and FACS analysis.
Human T-lymphocyte were stimulated in vitro with autologous CD14+ cells infected with US12-rVV, M-US12- rVV or control virus. Proliferation of specific CD8+ and CD4+ for viral proteins and the recombinant epitope were monitored by MHC-multimer and IFNg intracellular staining.

Results
- US12-rVV demonstrated MHC class-I downregulation.
- Kinetic analysis of MHC class-I downregulation indicated that this effect become most visible after 16-24h of infection.
- In HLA-A_2 positive cell lines , HLA-A2 downregulation with US12-rVV was partially compensated by presence of ER-Mart peptide in M-US12-rVV
- The absence of effect of US12-rVV on other surface molecules CD44, CD80 and MHC class II demonstrates that ICP47 effect is specific for MHC class-I molecule.
- Preliminary tests seem to confirm that CD8+ responses against viral epitopes (processed from vaccinia vector) are diminished when primed with US12-rVV.

Conclusion:
Recombinant vaccine expressing the HSV-US12 gene confirmed a diminished class-I recognition of native proteins from the viral vector. While helper-class-II properties should be conserved, this type of vector could thereby have a stronger immunogenic potential toward the recombinant ER-targeted class-I epitope. Such reagent could become of high relevance especially in multiple-boost vaccine protocol required in cancer immunotherapy.

Expansion of natural killer cells lacking broadly reactive inhibitory receptors post allogeneic hematopoietic cell transplantation

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Donor-derived alloreactive NK cells influence the prognosis post hematopoietic stem cell transplantation (HCT) for leukemia or lymphoma by enhancement of the graft-versus-leukemia (GvL) effect. Such donor-derived alloreactive killer cells can be generated *in vivo* after HCT if the donor expresses killer cell immunoglobulin-like receptors (KIR) such as KIR2DL1, KIR2DL2/3 or KIR3DL1 for which the recipient lacks HLA class I ligands such as the HLA-C group 2, group 1 or HLA-Bw4, respectively. We studied effector cells from 36 patient-donor pairs after allogeneic HCT (22 KIR-ligand mismatched, 14 KIR-ligand matched) using a novel 8-color flow cytometry panel which allowed us to characterize effector-cell populations without "broadly reactive" inhibitory receptors such as CD94/NKG2A or LIR1. $NKG2A^-$ $LIR1^-$ NK cells should play a predominant role in GvL because they are not inhibited by the expression by common HLA class I allospecificities (A, B and C) on their target cells. Thus, residual leukemia cells should be better stimulators of $NKG2A^-$ $LIR1^-$ NK cells in KIR-ligand mismatched transplants than in KIR-ligand matched transplants. In this study, transplanted patients who received KIR-ligand mismatched grafts had more $NKG2A^-$ $LIR1^-$ NK cells than patients who received KIR-ligand matched grafts. These NK cells, lacking broadly reactive inhibitory receptors, were functional killer cells since they expressed CD107a following incubation with K562 cells. Our data imply that there is activation and expansion of such potentially alloreactive NK cells *in vivo* following KIR-ligand mismatched HCT that have a protective role against disease recurrence.