an M2 polarized phenotype, associated with strong anti-inflammatory and immunosuppressive functions.
Due to the high capability of TAM to infiltrate solid tumours, here we investigated the effect of tolerance on macrophage migration, *in vitro* and *in vivo*.
For the *in vitro* study we assessed the capabilty of tolernat cells to responde to chemotactic stimuli both by western blot, measuring the phosphorylation of ERK protein, and by Boyden chambre. The air pouch mouse model help us to investigate *in vivo* the behaviour of macrophages from tolerant or non-tolerant animals during an inflammatory response.
Our study shows that LPS-tolerant macrophages maintain their capacity to respond to the chemotactic C5a complement factor, in terms of both cell migration and ERK1/2 phosphorylation. In contrast, LPS-tolerant macrophages did not respond to the chemokines CCL2 and CCL5. By using the air pouch model in mice treated systemically with LPS (in vivo tolerance) we further demonstrated a differential regulation of different leukocyte populations recruitment. In particular, a $F4/80^{+}C5aR\ (CD88)^{+}$ macrophage population was still recruited in response to C5a, in the air pouch of LPS-tolerant mice, supporting the functional activity of this pathway in *in vivo* tolerant conditions.
Future studies in our group will address the role of tolerance in driving selective accumulation of distinct polarized macrophage populations in pathological sites (eg. cancer, chronic inflammatory diseases). We speculate that selective recruitment of tolerant M2 populations may contribute to the extinction of the inflammatory response, thus contributing to restoring tissue homeostasis.

### 297 Therapy of murine HPV 16-associated TC-1 tumours: suppression of T regulatory and myeloid derived suppressor cells

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**Background:** Myeloid-derived suppressor cells (MDSC) interfere with tumour immunity, promote tumour growth by inhibiting tumour cell cytotoxicity and facilitate immune suppression and tumour progression. Immunosuppressive CD4+/CD25+ T regulatory (Treg) cells, which play a role in suppressing the function and proliferation of tumour-specific CD4+ and CD8+ T effector cells, represent another major mechanism by which tumours can escape immune recognition. To overcome these effects and improve the effect of immunotherapy, we used gemcitabine or all-trans-retinoic acid (ATRA) for induction of myeloid cell maturation in vivo, or administration of antiCD25 antibody (PC61) for depletion of Treg cells.
**Material and Methods:** Mice bearing established TC-1 tumour transplants (ca 0.02 cm2) were treated with gemcitabine (120 mg/kg, i.p.) or ifosphamide derivative CBM-4A (150 mg/kg, i.p.) in combination with ATRA (10 mg/kg, s.c., 2 cycles for 5 days) and cytokine (IL-2 or IL-12)-producing irradiated cellular vaccines (ca 40 mg cytokine/105 cells/ml/48 h). Treg cells were removed with PC61 Ab (antiCD25), day 4 after chemotherapy (i.p., 0.3 mg/mouse). For in vitro monitoring of immune response, ELISA and Elispot assays were used according to the manufacturer's instructions.
**Results:** The treatment with gemcitabine led to a significant tumour-inhibiting effect, to a decrease of the number of MDSC in the spleens of treated animals and to an improved effect of subsequent immunotherapy. The cytoreductive chemotherapy with CBM-4A, which resulted in strong upregulation and accumulation of immunosuppressive immature myeloid Gr-1+/CD11b+ cells in the spleens of the treated animals, was significantly decreased after subsequent therapy with ATRA. Moreover, this drug combination was able to improve subsequent immunotherapy with irradiated TC-1-IL-12 tumour vaccine. Further, it has been found that the removal of Treg cells with P61 Ab exhibited an additive effect to the subsequent immunotherapy with the IL-12-producing vaccine.
**Conclusions:** Taken together, removal of MDSC and Treg cells in vivo contribute to the boosted efficacy of cytokine-producing cellular vaccines for the therapy of early established tumour transplants minimized after chemotherapy and provided useful information for elaborating the optimal immunotherapeutic strategies for the treatment of HPV 16-associated tumours.
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### 298 Cartilage oligomeric matrix protein DNA vaccine in transgenic mice developing autochthonous mammary carcinomas

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**Background:** Through transcriptional profiling of mammary cancer appearing in mice transgenic for the Erbb-2 oncogene (BALB-neuT mice) (Cavallo et al. 2006) and bioinformatics meta-analyses on human genome-wide tumour transcription profile studies (Cavallo et al. 2007) new putative targets for anti-tumour vaccination were identified (Calogero et al. 2008). Among these, cartilage oligomeric matrix protein (COMP), that potently suppresses apoptosis in transformed cells by inducing the transcriptional up-regulation of survivin, one of the Inhibitor of Apoptosis Protein family members overexpressed in virtually all human cancer (Altieri 2003). COMP immunogenicity was tested in vaccination-protection assays in transgenic mice developing autochthonous mammary carcinomas: The Erbb-2 transgenic BALB-neuT mice and PyMT mice, transgenic for the polyoma middle T oncogene (Guy et al. 1992).
**Material and Methods:** COMP mouse transcript was cloned in pVAX1 vector (Invitrogen®, Milano, Italy). Mice were vaccinated with 50 mg COMP plasmid diluted in 20 μl sterile water with 0.9% NaCl injected twice with a two weeks interval into the quadriceps muscle, followed by electroporation using the CLINIPORATOR™ (Igea, Carpi, Italy). Mammary glands were palpated at weekly intervals to note tumour appearance. The induction of specific anti-COMP antibodies was evaluated in the sera using an ELISA kit (Kaminia®, Seattle, USA).
**Results:** BALB-neuT mice develop palpable mammary carcinomas starting by week 22 of age, while PyMT mice develop palpable carcinomas starting by week 11 of age. Mice were vaccinated when their mammary glands display atypical hyperplasia and in situ carcinomas (weeks 10 and 12 for BALB-neuT mice; weeks 6 and 8 for PyMT mice). In both BALB-neuT and PyMT mice vaccinated with COMP plasmid, a significant increase in tumour free survival and a significant decrease in tumour multiplicity were observed. Moreover, vaccinated mice developed specific anti-COMP antibodies in the sera.
**Conclusions:** This study show that COMP is a good target for antitumour vaccination, and that DNA vaccination targeting COMP is a suitable way for breaking immune tolerance.

### 299 Hypoxia inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing sustained hypoxia

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**Background:** Ischemia exists in many diseased tissues including arthritic joints, atherosclerotic plaques and malignant tumours. Macrophages accumulate in these sites and upregulate hypoxia-inducible transcription factors (HIFs) 1 and 2 in response to the hypoxia present.
**Material and Methods:** We used microarrays, qRT-PCR and cytokine release assay to study gene expression in hypoxic primary human macrophages, and then siRNA to block the hypoxic upregulation of HIFs 1α and 2α in order to study their role in regulating gene expression in these cells. We also investigated the role of NF-kB signaling in this phenomenon using both a synthetic inhibitor of p65 nuclear translocation and an adenoviral dominant negative inhibitor of IKKβ.
**Result:** We show that the expression of a number of genes, including the cell surface receptors, CXCR4 and GLUT1, and potent, tumour-promoting cytokines, VEGFA, interleukins 1β and 8 and adrenomedullin was upregulated in these cells in response to sustained (18h) hypoxia – and that this was regulated by both HIFs 1α and 2α. While hypoxia also stimulated the expression and/or phosphorylation of various proteins in the NF-κB signaling pathway, blockade of NF-kB signaling had little or no effect on the upregulation of these genes in sustained hypoxia.
**Conclusions:** These studies showed that both HIFs 1 and 2, but not NF-kB, are important transcriptional effectors regulating the responses of macrophages to sustained hypoxia. Further studies using experimental mouse models are now warranted to investigate the role of such macrophage responses in the progression of various diseased tissues like malignant tumours.

### 300 A novel immunotherapy approach for B-CLL by use of Chimeric TCR

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**Background:** B-cell chronic lymphocytic leukemia (B-CLL), the most common form of leukemia in adults in Western countries, is characterized by a progressive accumulation of mature $CD19^{+}CD5^{+}CD20^{dim}$ B lymphocytes that over-express the B-cell activation marker CD23. Here we cloned and expressed in T lymphocytes a novel chimeric antigen receptor (CAR) that targets the CD23 antigen (CD23.CAR).