reference gene expression value and HER2 gene expression value in the normal breast tissue. A ratio $\geqslant$2.6 was regarded as being positive for HER2 amplification. Pearson and partial correlation analyses were done and $p < 0.05$ was considered statistically significant.

Results: Comparison of the assays was detailed in Table 1. One case of chromosome 17 monosomy and 2 cases of chromosome 17 polysomy were found. IHC had significant correlation with only FISH ($P = 0.001$); other amplification methods revealed significant correlation with each other (qRT-PCR & CISH, $P = 0.000$; qRT-PCR & FISH, $P = 0.035$; FISH & CISH, $P = 0.012$).

Conclusions: FISH seems to be best standard assay for determination of HER2 status but other amplification methods must be prospectively tested in large tumor samples and clinical trials. Clinical impact of polysomy 17 must also be evaluated.

Table 1: Comparison of assays for the assessment of HER2 protein expression and gene amplification.

IHC N = 54		FISH N = 54		CISH N = 54		qRT-PCR N = 54	
Score	No. of pts	Amplified	Not Amplified	Amplified	Not Amplified	Amplified	Not Amplified
0	25	0	25	4	21	2	23
1(+)	10	1	9	4	6	4	6
2(+)	8	0	8	2	6	2	6
3(+)	11	7	4	4	7	3	8

N: No. of pts

1046 POSTER

Does lactate have an impact on enzyme activity?

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Background: We still do not fully understand all roles of lactate in tumor growth. At present it is postulated that lactate serves as a whole-organism metabolic signal. Following this hypothesis we can suggest that lactate like a hormone can modulate enzymes activities. Adenosine deaminase (ADA) is a key enzyme of purine nucleotides metabolism. Increased lactate concentration and ADA's activity in tumor tissues is a characteristic feature for neoplasm.

The aim of the current investigation was to determine if lactate has an impact on activity of ADA in erythrocytes.

Materials and Methods: The different amounts of Lithium lactate have been added to red blood cells suspended in buffer-solution (pH 7.4). The range of lactate concentration was 7.5 to 30 mM whilst pH was supported on constant level. An incubation period with lactate was 10 minutes. Then erythrocytes have been hemolysed and activity of ADA was determined spectrophotometrically.

Results: We found that the dependence of activity of ADA on lactate concentration has extremum-like shape. The 3-times growth of ADA activity was observed upon concentration of lactate from 7.5 mM to 20 mM. Further elevation of lactate concentration led to decreasing ADA activity to initial level (obtained in control tube without lactate).

Table. Activity of ADA in erythrocytes after lactate addition

	Lactate, mM						
	0, control	7.5	10	15	20	25	30
ADA*, nmol/min?mg	6.2±0.7	8.2±0.3	9.7±0.4	11.8±0.6	20.8±1.9	13.2±0.3	9.0±0.2

* $p < 0.005$

Conclusion: The lactate impact is not a simple effect. Our results suggest that lactate can definitely modulate ADA activity and we can reject effects concern with genes expression due to erythrocytes metabolism features. Such complicated impact can be described by two different lactate influence (activation and inhibition) with different dose-sensitivity to lactate.

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Extracellular matrix of glioblastoma inhibits polarization and transmigration of T cells: a role of tenascin-c in immune suppression

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Background: A tumour-bearing host can raise a tumor-specific T cell response. However, dense accumulations of T cells are often found in peritumoral areas, which could reduce the efficiency of contact-dependent lysis of tumour cells. The mechanisms contributing this phenomenon remain unclear. In this study we evaluated the effect of extracellular matrix (ECM) of tumor cells on T cell migration.

Methods: Transwell system was used to measure the transmigration of Jurkat, Molt-4, and primary T cells through cell monolayers and ECM of MCF-7, U-87MG, U-118MG, U-373MG, and HepG2 cells. Morphological changes and protein kinase expressions of T cells on cell monolayers and ECM of the tumour cells were detected by time-lapse microscopy and western blotting, respectively. Location of the protein kinase in migrating T cells was identified by confocal microscopy. Components of tumor ECM were verified by RT-PCR. The individual component was downregulated by shRNA.

Results: The transmigration rate of T cells measured for U-87MG, U-118MG and U-373MG (glioma cells) was consistently low as compared with that for HepG2 or MCF-7 cells. Jurkat T cells showed reduced ameba-like shape formation and delayed ERK activation when they were in contact with monolayers or ECM of glioma cells as compared with those in contact with HepG2 and MCF-7 cells. Glioma cells, but not MCF-7 and HepG2 cells, expressed tenascin-C. Knocking down the tenascin-C gene converted glioma cells to a transmigration-permissive phenotype for Jurkat T cells regarding ERK activation, transmigration, ameba-like shape formation, and phospho-ERK locating at the leading edge of migrating cells. Exogenous tenascin-C protein reduced the amoeba-like shape formation and transmigration of Jurkat T cells through MCF-7 and HepG2 cell monolayers. In addition, high level of tenascin-C was visualized immunohistochemically in human glioma tumor tissues. $CD3^+$ T cells were detected in the boundary tumor area stained strong positive for tenascin-C.

Conclusions: ECM produced by tumor cells could directly regulate T cell migration. Glioma cells actively paralyze T cell migration by the expression of tenascin-C, representing a novel immune suppressive mechanism achieved through tumour ECM.

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Requirement of fully activated dendritic cells for elicitation of potent anti-tumour immune responses in cancer patients with impaired immunity

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A number of clinical trials of cancer immunotherapy have been done so far. None of them has reported satisfactory outcomes. It is mainly because anti-tumor immune responses are hardly activated in cancer patients whose immunity is generally impaired. In patients with metastatic cancer, immunosuppressive factors such as transforming growth factor (TGF)-beta, vascular endothelial growth factor (VEGF) and interleukin (IL)-10 produced by tumor cells or tumor-stromal cells abrogate the maturation of dendritic cells (DC) which have a crucial role in eliciting potent anti-tumor cellular immune responses. In this study, we established a strategy for inducing fully activated mature DCs *ex vivo*. Immature DCs prepared from peripheral blood monocytes by a conventional culture supplemented with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4, were maturated by further 48 h-culture in the presence of anti-CD40 antibody and penicillin-killed streptococcus pyogenes (OK432). The induced DCs showed higher levels of CD80, DC86 and HLA-DR expression when compared with those in mature DCs which were prepared by a conventional strategy using tumor necrosis factor (TNF)-alpha or lipopolysaccharide (LPS). Furthermore, the fully activated DCs continued to produce a large amount of T-helper 1 type cytokines, i.e., interferon (IFN)-gamma and IL-12 for at least 3 days. In contrast, the conventional mature DCs demonstrated the predominant production of T-helper 2 type cytokines and production of low levels of T-helper 1 type cytokines. The cytokine production was expired within 3 days. Cytotoxic T lymphocytes (CTL) that were induced from peripheral blood lymphocytes of patients with metastatic breast cancer *in vitro* using fully activated mature DCs as a stimulator showed a strong antigen-specific killing activity of target cells, whereas CTLs induced using conventional mature DCs did not kill the target. Therefore, we expect that the fully activated DCs are applicable to DC-based vaccine for cancer patients with impaired anti-tumor immunity.