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# Session 2: Cancer – II

*Wednesday 10 May, 2006. Moderators: Zdenka L. Jonak and Peter Vollmers*

[11.30-12.00]
**Antibody microarrays: Technology and analysis of serum proteomes from cancer patients**
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Antibody-based microarray is a high-throughput technology with great potential in global proteome analysis and protein expression profiling. This tool will provide new opportunities for diagnostics, biomarker discovery, drug target identification and insights into disease biology. In our laboratory, we have successfully developed a high-performing human antibody microarray technology platform based on recombinant antibody fragments. In particular, the single-frame work concept in scFv design (SinFab) has been developed for microarray application, yielding >12 month on-chip-stability, assay sensitivity in the fM range, and direct analysis of labeled complex proteomes. This antibody microarray set-up has been used in a variety of applications, such as serum/cell proteome analysis of low as well as high abundant analytes. Recent projects focusing on cancer proteomics, using SinFab microarrays, have been performed, using sera from different patients suffering from solid tumors, such as e.g. pancreatic carcinomas, breast carcinomas, gastric adenocarcinomas etc. The optimized antibody microarray platform, as well as data from the clinical proteome analysis, will be presented.

[12.00-12.20]
**Endogenous antiganglioside IgM titers: A potential biomarker of malignancy**
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Gangliosides are amphophilic sialoglycolipids overexpressed on human tumor cells and released into the circulation during the necrosis that accompanies cell proliferation. Because circulating gangliosides have an immunosuppressive effect that promotes the progression, invasion, and angiogenesis of human cancers, they are promising targets for antibody-mediated passive and active specific immunotherapies. In previous studies of preoperative and postoperative serum specimens from patients with advanced colon cancer, we demonstrated that cryosurgical ablation (mechanically induced necrosis) of hepatic metastases was followed by an increase in the serum level of total gangliosides, and subsequently by selective augmentation of antiganglioside IgM titers. We hypothesized that necrosis during active tumor proliferation also would increase titers of IgM antibodies against gangliosides of colon cancer and other solid malignancies. We further hypothesized that this increase would be directly related to tumor burden or stage of disease.

In sarcoma, IgM titers differed with high- and low-grade tumors; by univariate and multivariate analyses, augmented IgM titers against selected gangliosides were correlated with survival of patients above 50 years of age. In prostate cancer, IgM titers against selected tumor-associated gangliosides were significantly higher in age-matched patients with organ-confined disease (stages T1 & T2) compared to unconfined disease (T3 & T4), benign prostatic hyperplasia or no prostate disease. In ovarian cancer, antiganglioside IgM titers were significantly higher in ascitic fluid, suggesting that release of gangliosides into the tumor cell's microenvironment may induce production of IgM. Finally, in patients with primary melanoma and tumor-negative regional nodes, antiganglioside titers distinguished patients with lesions of 1.00 to 1. 49 mm, from those with lesions of 1.50 to 2.00 mm.

These findings for various solid malignancies

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strongly support a role for antiganglioside IgM titers as potential biomarkers of human cancer. Because endogenous levels of antiganglioside IgM in healthy individuals decline significantly after the age of 50 years, IgM antibody titer might be particularly useful as a screening tool in the older population.

We have developed a model to show how tumor progression, necrosis, and shedding of cell-surface gangliosides might elicit the production of interleukin-6, which in turn could stimulate a T-cell independent IgM response that produces polymeric antiganglioside antibodies with no J chain and without propensity for class switching.

[12.20-12.40]

**Anti-tumor effects of human monoclonal antibody HMMC-1 on lymph node metastasis of endometrial cancer**

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*Keywords:* human monoclonal antibody, endometrial cancer, lymph node metastasis

*Aims:* Lymph node metastasis is a poor prognostic factor in gynecologic cancers; yet the study of its basic pathophysiology has been hampered by the lack of an *in vivo* model. We thus established a mouse model of lymph node metastasis using orthotopic implantation of an endometrial cancer cell line. On the other hand, the human monoclonal antibody HMMC-1 specifically recognizes gynecologic cancers and demonstrates positive immunohistochemical staining in greater than 50% of endometrial cancer surgical specimens; interestingly, positive cases also demonstrate a high rate of lymph node metastasis. The aim of this investigation was to study the correlation of HMMC-1 antigen expression levels and lymph node metastasis potential. In addition, we examined the anti-tumor effects of HMMC-1 antibody administration to lymph node metastasis in an *in vivo* lymph node metastasis mouse model.

*Methods:* 1) Lymph node metastasis potential of HMMC-1 high and low-expression endometrial carcinoma Hec 1A cells were examined based upon orthotopic implantation to nude mice. 2) The anti-tumor effects of HMMC-1 antibody administration (100 μg/wk, 8 wks) were examined in the *in vivo* lymph node metastasis mouse model based upon HMMC-1 high-expression Hec 1A cells.

*Results:* 1) HMMC-1 high-expression Hec 1A cells formed retroperitoneal lymph node metastasis in 80% (16/20) of orthotopically implanted mice whereas low-expression Hec 1A cells formed metastasis in 10% (1/10) of mice. 2) HMMC-1 antibody administration resulted in a significant decrease in metastatic lymph node tumor volume (n=8) compared with control (n=8).

*Conclusions:* Our results suggest that HMMC-1 antigen levels are positively correlated with lymph node metastasis potential in endometrial cancer. In addition, HMMC-1 antibody demonstrated an inhibitory effect *in vivo* towards retroperitoneal lymph node metastasis. In conclusion, the anti-tumor effects of the human HMMC-1 antibody suggest the possible use of this antibody as a new therapeutic strategy towards lymph node metastasis in endometrial cancers

[12.40-13.00]

**Anti-vascular endothelial growth factor receptor 1 antibody as an antagonist for cancer therapy through targeting cancer cells and tumor angiogenesis**

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*Introduction:* The VEGF/VEGF receptor pathways are essential for regulation of tumor angiogenesis and have been considered a therapeutic target for treatment of tumors and angiogenesis-associated diseases. Vascular endothelial growth factor receptor 1 (VEGFR-1) plays important roles in promotion of tumor growth through mediating cellular functions in tumor vascular endothelium and cancer cells. Blockade of VEGFR-1 activation have been shown to inhibit pathological angiogenesis and tumor growth, implicating VEGFR-1 as a potential therapeutic target for treatment of cancer. Thus, we developed a VEGFR-1 antagonist human monoclonal antibody (mAb) designated as IMC-18F1 and evaluated therapeutic potentials of the antibody in preclinical experimental models.

Table 1
The binding and blocking characteristics of anti-human VEGFR-1 antibodies

| Clone | Kinetics of Anti-VEGFR-1 Antibodies [a] | | | Binding Activity [b] (ED50, nM) | Blocking Activity [c] (IC50, nM) |
|---|---|---|---|---|---|
| | $K_{on}$ $10^5$ $M^{-1}s^{-1}$ | $K_{off}$ $10^{-5}$ $s^{-1}$ | $K_d$ $10^{-11}$ M | | |
| 6F9 | 11.1 ± 1.26 | 7.38 ± 2.01 | 6.9 ± 0.36 | 0.145 ± 0.03 | VEGF-A: 1.05 ± 0.03<br>VEGF-B: ND [d]<br>PlGF: 0.81 ± 0.05 |
| 13G12 | 9.58 ± 1.63 | 11.05 ± 3.06 | 12.4 ± 0.53 | 0.303 ± 0.02 | VEGF-A: 0.99 ± 0.02<br>VEGF-B: ND<br>PlGF: 0.73 ± 0.04 |
| 15F11 | 10.16 ± 2.84 | 7.16 ± 2.21 | 7.0 ± 0.23 | 0.304 ± 0.01 | VEGF-A: 0.82 ± 0.03<br>VEGF-B: ND<br>PlGF: 0.48 ± 0.01 |
| IMC-18F1 | 8.1 ± 2.4 | 4.29 ± 1.05 | 5.4 ± 0.3 | 0.112 ± 0.04 | VEGF-A: 1.04 ± 0.01<br>VEGF-B: 0.43 ± 0.01<br>PlGF: 0.38 ± 0.02 |

[a] The values of kinetics of the antibodies were calculated from the data determined by BIAcore analysis.
[b & c] The ED 50 and IC50 values were calculated from the data analyzed using GraphPad Prism® software. Binding activity was determined by measuring ability of the antibodies binding to rhu-VEGFR-1 in ELISA. Blocking activity was determined by measuring ability of the antibodies preventing rhu-VEGFR-1 from binding to VEGF-A, VEGF-B or PlGF in ELISA.
[d] ND: not determined.

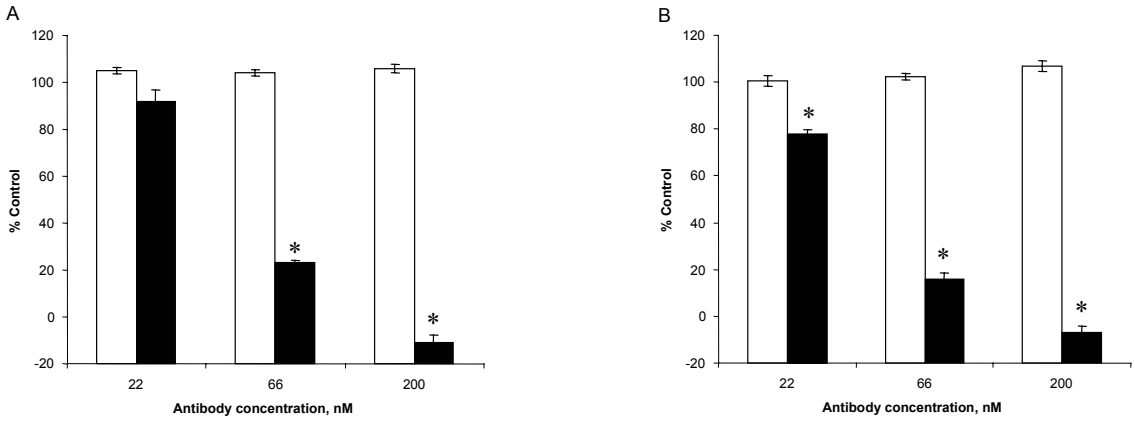


Fig. 1. IMC-18F1 inhibits ligand-induced in vitro growth of VEGFR-1+ breast cancer cells. IMC-18F1 significantly inhibited proliferation of the DU4475 tumor cells stimulated with VEGF-A (A) or PlGF (B) in a dose dependent manner. The data are represented as percentage of inhibition calculated by % control. Asterisks indicate statistic significance ($p<0.05$) as comparison between IMC-18F1 (closed bar) and isotype control antibody (opened bar) treated groups.

*Methods: Anti-VEGFR-1 Monoclonal Antibodies.* Human IgG transgenic mice were used for generation of anti-VEGFR-1 mAbs. Lewis rats were used for generation of anti-mouse VEGFR-1 mAbs. Hybridoma generation was performed using a standard hybridoma technology. Anti-VEGFR-1 specific antibodies were identified using solid phase binding and blocking assays. Inhibitory activity of IMC-18F1 was assessed in cell-based kinase and growth assays. IMC-18F1 is a human IgG 1 kappa anti-human VEGFR-1 mAb. MF1 is a rat IgG 1 anti-mouse VEGFR-1 mAb.

*Tumor Cell Growth Assay:* DU4475 cells were seeded in 96-well plates and incubated in serum-free conditions for 18 hrs, then cultured in 1% serum conaining medium with IMC-18F1 or isotype control

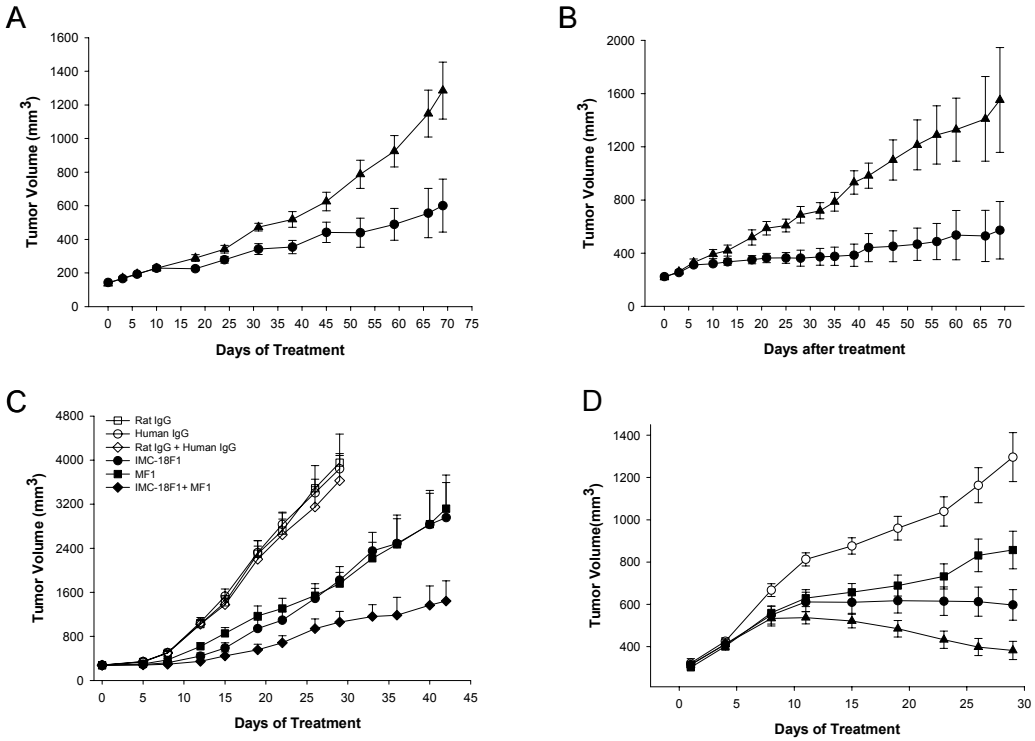


Fig. 2. Inhibition of human breast tumor xenografts. Monotherapy with IMC-18F1 (closed circle) significantly inhibited the growth of MDA-MD-435 (A), MDA-MD-231 (B) and DU4475 (C) xenografts compared to controls vehicle (PBS, closed triangle, A and B) or 20 mg/kg human normal IgG (open circle, C). C. VEGFR-1 antibody-mediated inhition of human VEGFR-1 in tumor xenografts and murine VEGFR-1 in vascular endothelium enhanced the antitumor effect. Co-treatment with IMC-18F1 and MF1 resulted in a significantly increased antitumor activity ($p<0.01$) compared to monotherapy with IMC-18F1 or MF1 in the DU4475 xenograft model. D. VEGFR-1 antibody treatment increased antitumor efficacy of cytotoxic agent against human breast cancer. Mice bearing established MDA-MB-231 tumor xenografts were treated with 20 mg/kg IMC-18F1 and 40 mg/kg MF1 three times each week (closed square), 3 mg/kg doxorubicin twice a week (closed circle), or IMC-18F1 and MF1 plus the cytotoxic agent (closed triangle) that resulted in a significantly enhanced antitumor efficacy ($P<0.02$) compared to monotherapy with VEGFR-1 antibodies or cytotoxic agent alone. Mean tumor volume ± SEM is plotted for n =12 per group.

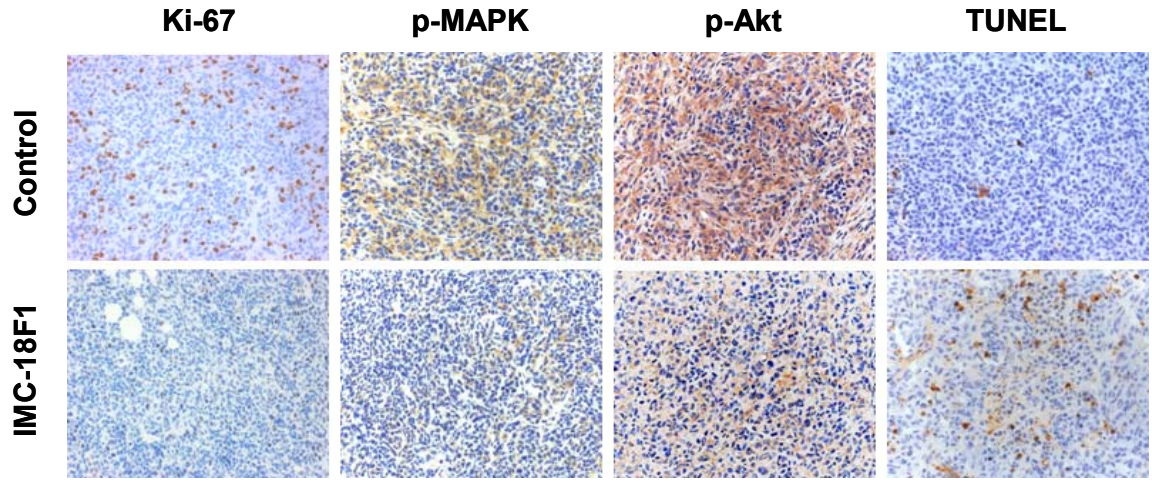


Fig. 3. Histology analysis of cell proliferation and intracellular activities of p-MAPK, p-Akt and apoptosis in IMC-18F1-treated MDA-MB-231 xenograft tumors. A significantly reduced activity of proliferation, p-MAPK, p-Akt and increased apoptosis were found in IMC-18F1 treated tumors compared to the controls of human IgG-treated tumors. Representative tumor sections are shown.

Table 2
Evaluation of VEGFR-1 antibody combined with cytotoxic agents compared with monotherapies against established human breast tumor xenografts

| Agent [a] | Dose (mg/kg) | Mean Tumor Volume ($mm^3 \pm$ SEM) | % T/C [b] (day) | P-value (RM-ANOVA) |
|---|---|---|---|---|
| Control | NA | 1296 ± 116 | NA | |
| IMC-18F1&MF1 | 20/40 | 857 ± 89 | 69 (29) | 0.005 } vs. control |
| 5FU/LV | 125/65 | 919 ± 73 | 73 (29) | 0.008 } vs. control |
| IMC-18F1&MF1 + 5FU/LV | 20/40+125/65 | 648 ± 45 | 51 (29) | <0.0001 } vs. control<br><0.02, vs. Abs [c]<br>0.0004, vs. 5FU/LV |
| IMC-18F1&MF1 | 20/40 | 857 ± 89 | 69 (29) | 0.005 } vs. control |
| Doxorubicin (Dox) | 3 | 597 ± 72 | 54 (29) | <0.0001 } vs. control |
| IMC-18F1&MF1 + Dox | 20/40+3 | 382 ± 43 | 30 (29) | <0.0001 } vs. control<br><0.0001, vs. Abs [c]<br>0.01, vs. Dox |

[a] IMC-18F1 and MF1 were administered i.p. three times each week. 5FU/LV were given i.p. once a week. Doxorubicin was given i.p. twice weekly.
[b] T/C, tumor growth inhibition, where T is the mean tumor volume of treated group and C is the mean tumor volume of control group on the designated day.
[c] IMC-18F1&MF1.

IgG in the presence of 50 ng/ml of VEGF or 200 ng/ml of PlGF for 48 hrs. Viable cells were counted in triplicate using a Coulter cytometer. Cell viability was calculated as a percentage of the control.

*Tumor Xenograft Models:* Athymic nude mice were injected subcutaneously with 2 x $10^6$ of DU4475 cells, 5 x $10^6$ of MDA-MB-231 or MDA-MB-435 cells mixed in Matrigel. For the antibody combination treatment, mice bearing established tumors (200 $mm^3$) were treated with 20 mg/kg IMC-18F1 twice weekly and 40 mg/kg MF1 three times per week. For combination treatment with antibody and chemotherapy, mice bearing tumor (350 $mm^3$) were administered i.p. with a cocktail of 20 mg/kg IMC-18F1 and 40 mg/kg MF1 one or two day(s) before chemotherapy with 125 mg/kg 5-Fluorouracil and 62 mg/kg leucovorin (5-FU/LV) q7d or 3 mg/kg doxorubicin twice a week. Mice in control groups received vehicle or normal IgG. Tumor volumes were calculated using the formula p/6 (w1 X w2 X w2)], where "w1" represents the largest tumor diameter and "w2" represents the smallest tumor diameter.

*Immunohistochemical analysis of VEGFR-1 expression in human tumor specimens and VEGFR-1 antibody-treated tumor xenografts:* Frozen sections of human invasive ductal breast carcinoma of varying pathological stages were stained for VEGFR-1 expression using a non-neutralizing anti-huVEGFR-1 mAb and the EnVision+ Mouse kit (DAKO). Paraffin-embedded tumor xenografts were evaluated for markers of cell proliferation, survival, and apoptosis. Antibodies to Ki-67, phospho-specific p44/42 MAPK and phospho-specific Akt were used as primary antibodies. The EnVision+ System for rabbit antibodies was used with 3,3' diaminobenzidine as the chromagen. Tumor apoptosis was assessed by TUNEL assay using ApopTag® Peroxidase In Situ Apoptosis Detection Kit. Immunostaining were analyzed and imaged using an Axioskop light microscope and Axiocam digital camera.

*Statistical analysis:* Tumor volume data were analyzed using repeated-measures ANOVA (RM-ANOVA) to determine the significant differences in tumor sizes among treatments, time points, and treatment-time interactions. Comparisons of in vitro tumor cell growth between treatment and control were conducted using the two-tailed Student's t test. P value of less than 0.05 was considered to be statistically significant.

*Results:* VEGFR-1 expression was detected in cancer cells, tumor vascular endothelium and myoepithelium layers in human breast and colon carcinoma specimens. VEGFR-1 is also present in breast, colon and pancreatic carcinoma cell lines tested. A panel of neutralizing human mAbs specific to VEGFR-1 was generated from the human Ig

transgenic mice (Table 1). One clone IMC-18F1 was identified to have strongest affinity binding ($K_D$ = 54 pM) to the extracellular domain of VEGFR-1. The antibody efficiently blocked binding of VEGF-A, VEGF-B and PlGF to VEGFR-1 with IC50 of 1.04, 0.43 and 0.38 nM, respectively. IMC-18F1 inhibited PlGF-induced intracellular activation of VEGFR-1 and MAP kinase signaling, and prevented VEGF-A and PlGF-stimulated *in vitro* growth of breast cancer cells (Figure 1). Treatment with IMC-18F1 significantly suppressed the growth of human breast tumor xenografts through inhibition of MAPK and Akt activation and proliferation in tumor cells and induction of tumor cell apoptosis (Figure 2 & 3). Remarkably, selective inhibition of human VEGFR-1 expressed in human breast tumor xenografts by IMC-18F1 and murine VEGFR-1 in tumor vasculature by anti-mouse VEGFR-1 antibody MF1 resulted in an increased antitumor effect. Furthermore, IMC-18F1 and MF1 treatment enhanced the antitumor efficacy of cytotoxic agents against tumor xenografts (Table 2, Figure 3).

*Conclusion:* Functional VEGFR-1 conveys intracellular signalings promoting survival and proliferation in cancer cells and modulating tumor vascularization, suggesting that VEGFR-1 is a potential therapeutic target for cancer intervention. IMC-18F1 has a therapeutic potential for monotherapy and combination treatment with cytotoxic agents in patients with cancer.

*References*

[1] Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 2005; **23**: 1011-27

[2] Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin DJ, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S and Lyden D. VEGFR1$^+$ hematopoietic bone marrow progenitors initiate the premetastatic niche. Nature. 2005; **438**: 820-827.

[3] Fan F, Wey JS, McCarty MF, Belcheva A, Liu W, Bauer TW, Somcio RJ, Wu Y, Hooper A, Hicklin DJ, Ellis LM. Expression and function of vascular endothelial growth fac tor receptor-1 on human colorectal cancer cells. Oncogene. 2005; **24**: (16):2647-2653.

[4] Wang ES, Teruya-Feldstein J, Wu Y, Zhu Z, Hicklin DJ, Moore MA. Targeting autocrine and paracrine VEGF receptor pathways inhibits human lymphoma xenografts in vivo. Blood 2004; **104**: 2893-902.

[5] Hattori K, Heissig B, Wu Y, Dias S, Tejada R, Ferris B, Hicklin DJ, Zhu Z, Bohlen P, Witte L, Hendrikx J, Hackett NR, Crystal RG, Moore MA, Werb Z, Lyden D, Rafii S. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. Nat Med 2002; **8**: 841-849.

[6] Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, Nagy JA, Hooper A, Priller J, De Klerck B, Compernolle V, Daci E, Bohlen P, Dewerchin M, Herbert JM, Fava R, Matthys P, Carmeliet G, Collen D, Dvorak HF, Hicklin DJ, Carmeliet P. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat Med 2002; **8**: 831-840.

[7] Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, Shipley JM, Senior RM, Shibuya M. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell 2002; **2**: 289-300.

[8] Hiratsuka S, Maru Y, Okada A, Seiki M, Noda T, Shibuya M. Involvement of Flt-1 tyrosine kinase (vascular endothelial growth factor receptor-1) in pathological angiogenesis. Cancer Res 2001; **61**: 1207-1213.

[13.00-13.20]

**Recruitment of NK cells to kill tumor cells**

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We have established a large naïve IgM-based antibody library from which we selected an antibody that was specific for the A isoform of the Fc□III receptor, which is found on the surface of natural killer cells. It showed no reactivity towards the B isoform which is present in relatively large numbers on other immune cells, particularly granulocytes. Using calcium mobilisation assays we were able to show that the antibody was able to efficiently activate NK cells. After combining it with antibodies in a tetravalent bispecific format (tandab technology) that targeted tumor cells, we were able to achieve a highly effective cell lysis.