In the Dutch Gastric Cancer Group trial 711 patients that were treated with curative intent were randomized between D1 and D2 lymph node dissection. After a median follow up of 11 years there was no survival difference (30% vs. 35%; $p = 0.53$). Morbidity (25% vs. 43%; $p < 0.001$) and mortality (4% vs. 10%; $p = 0.004$) however, were significantly higher in the D2 group [4]. In the British MRC trial 400 gastric cancer patients were also prospectively randomized between D1 and D2 lymph node dissection [5]. Five year survival was 35% in the D1 and 33% in the D2 group; morbidity was 28% and 46% respectively, mortality was 6.5% for D1 and 13% in D2. Since these two trials were published a lot of debate has been generated about two topics. First of all, since subgroup analyses have indicated a trend for better survival in N2 patients after a D2 dissection, the question has risen whether there is a role for D2 resections in this subset of patients. Furthermore, there is considerable debate about the role of routine splenectomy and resection of the pancreatic tail in order to facilitate a D2 resection. It is hypothesized that in performing a D1 dissection without splenectomy and resection of the pancreatic tail, together with dissection of at least 15 (N1 and N2) nodes, a so-called D1 over (D1+) resection, can result in better outcome [6,7].

In 2005 final results of the MAGIC-study on perioperative chemotherapy have been presented [8]. In this large multicenter study patients were randomized between surgery only and 3 cycles preoperative ECF (epirubicin, cisplatin, 5-FU) followed by surgery and then another 3 cycles of ECF chemotherapy. This regimen resulted in a 10% higher resectability rate and a significant survival benefit of 13% (23% vs. 36% at 5 years). It should be noted that 80% underwent surgical resection, and that 66% of the patients commenced the postoperative chemotherapy and 42% completed the entire treatment. In addition, 50% of patients who completed preoperative chemotherapy and surgery, also completed postoperative treatment. The main reason (70% of the patients) for not starting postoperative chemotherapy was disease progression or patient choice (Cunningham, ASCO GI 2006). Despite this disappointing number of patients undergoing systemic treatment, perioperative chemotherapy with ECF may be considered as a new standard of treatment in operable gastric cancer.

In a Cochrane review of randomized trials in advanced gastric cancer highest survival rates were achieved with anthracyclines, cisplatin and 5-FU, both independently and in combination (Cochrane Library, 2005). Within these combinations ECF proved to be tolerated best. However, the use of continuous infusional 5-FU is considered cumbersome, because it requires the implantation of central venous catheter devices and the use of portable infusion pumps, which are associated with complications such as thrombosis and wound infection. Capecitabine, a prodrug and oral analogue of 5-FU, is believed to mimic continuous infusion of 5-FU and has demonstrated to be at least equally effective in tumor control and to be less toxic than intravenous 5-FU in patients with stage III and IV colon cancer.

In 2001, with the introduction of postoperative combined chemoradiotherapy for the first time a substantial improvement in survival and locoregional control has been described. In the SWOG/ Intergroup 0116 trial 556 patients were prospectively randomized between surgery only and surgery plus postoperative chemoradiotherapy. Radiotherapy consisted of 45 Gy in 25 fractions in five weeks. The chemotherapy regimen consisted of three cycles of 5-fluorouracil and leucovorin according to the Mayo regimen perioperatively and two shortened courses during radiotherapy. An impressive increase in median overall survival was obtained in the chemoradiotherapy group; 36 months versus 27 months in the surgery only group. Furthermore relapse free survival was prolonged from 19 months in the surgery only arm to 30 months in the chemoradiotherapy arm. It was thus shown that in gastric cancer too, the advantage in combining modalities is the ability to address both locoregional and systemic disease simultaneously. This postoperative chemoradiotherapy regimen has become standard treatment in the USA; nevertheless this study has been criticized because of suboptimal surgery, concerns about toxicity, an outdated chemotherapy regimen and suboptimal radiotherapy techniques. Indeed, 54% of all patients underwent a D0 lymph node dissection, which in it self could be one factor in undermining survival.

Taken the abovementioned pivotal MAGIC and SWOG/Intergroup studies together, the important question that needs to be answered is whether postoperative chemoradiotherapy improves survival and/or locoregional control in patients that receive neoadjuvant chemotherapy followed by a D1+ gastric resection. We therefore conduct a prospective randomized multicenter phase III trial addressing this important question. To ascertain patient compliance and improve patient selection/ treatment tailoring, we plan to incorporate validated prognostic and predictive tests, such as Maruyama Index and nomogram for gastric cancer. In the chemoradiotherapy arm state-of-the-art 3D-conformal or Intensity-Modulated RadioTherapy (IMRT) should be a minimal requirement in order to limit normal tissue toxicity, in particular kidney damage. The chemotherapy schedule in both arms should be effective and safe. The combination of epirubicin, cisplatin and capecitabine fulfils these requirements. An optimized chemoradiotherapy schedule with radiosensitizing drugs during the entire radiotherapy treatment has been established with daily cisplatin and capecitabine in our phase I−II study.

- A phase III study which randomizes between preoperative chemotherapy (3 courses of epirubicin, cisplatin and capecitabine (ECC)) and D1+ gastric surgery followed by postoperative chemotherapy (another 3 courses of ECC) or chemoradiotherapy. Chemoradiotherapy consists of 45 Gy radiotherapy in 25 fractions with concurrent capecitabine and cisplatin (protocol available upon request).

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Special session (Wed, 26 Sep, 13:30−14:30)
New developments in clinical functional imaging

139 INVITED

Angiogenesis imaging

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Background: Angiogenesis, the growth of new blood vessels, plays an important role in reproduction and wound healing as well as in tumor progression. Non invasive imaging of angiogenesis can help in preclinical drug discovery and development and can also provide biomarkers during clinical monitoring of targeted antiangiogenic therapy.
Materials and Methods: Over the last years multiple approaches for imaging angiogenesis were developed for the various imaging modalities, providing structural, functional and molecular markers of the process.
Results: Imaging angiogenesis includes nowadays a large family of methods providing structural information on blood volume, vessel diameter and tortuosity. Functional information revealed by imaging includes blood flow and perfusion, vessel permeability and vasoreactivity. Lastly molecular imaging allows to probe changes in the composition and enzymatic activity in the extracellular matrix, expression of specific cell surface markers on endothelial cells, and imaging methods for following the recruitment of vascular and perivascular precursor cells as well as in vivo detection of gene expression.
Conclusions: Over the last decade multiple imaging approaches were developed to detect angiogenesis, thus complementing the efforts for therapeuric intervention. Clinical translation of these imaging approached could help tailor antiangiogenic therapy and provide early mechanism based markers for response.

140 INVITED

Two-photon imaging of tumour invasion

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Multiphoton microscopy has defined standards for 3D fluorescence and higher harmonic generation analysis of cells and tissue structures in vitro and in vivo. Compared to single-photon excited confocal microscopy, two-photon microscopy utilizes near-infrared (NIR) excitation generating twice to multi-fold enhanced tissue penetration, reduced light scattering and

minimized phototoxicity and photobleaching at out-of-focus regions, yet preserves submicron spatial resolution and subcellular detail of cell and tissue structures.
Using invasive HT-1080 fibrosarcoma xenografts in the dorsal skin-fold chamber in nude mice, we here show the dynamics of tumor growth, neoangiogenesis, and tumor invasion into the adjacent tissue microenvironment. Using fluorescent labels, not only single cells but also extensively invading collective cell strands were reconstructed to move along and around preexisting blood and lymphatic vessels, not however neovessels. Using dual-color cells expressing Histone-H2B/eGFP in the nucleus and cytoplasmic RFP, the combined dynamics of collective invasion and mitotic activity defines the hallmarks of 'invasive growth'. In future studies, time-resolved two-photon microscopy will allow to gain novel insight into the mechanisms cancer progression, regression, and persistence during experimental therapy.

141 INVITED

The use of iron particles in MRI

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Pelvic lymph node metastases have a significant impact on the prognosis of patients with malignancies. In prostate cancer, for example, even micro metastases in a single node rule out surgical cure by the available treatment protocols. For bladder cancer lymph node metastasis are also significant. More than 5 lymph node metastasis or extra capsular growth precludes curative surgical treatment. Thus, the status of the lymph nodes largely dictates the management of the primary tumour. Surgical open pelvic lymph node dissection (PLND) considered being the only reliable method for assessing lymph node status is an invasive procedure associated with potential complications and side effects. A noninvasive, reliable method for detecting and staging nodal metastasis would reduce unnecessary surgery. Routine cross-sectional imaging modalities like CT and MRI lack the desired sensitivity in identifying metastases as they largely rely on size criteria only, and small metastases in normal size nodes can be missed. Moreover, differences in signal intensity on MR images between normal and cancerous nodes as well as gadolinium enhancement have also proven to be unreliable. Ultra small super paramagnetic iron oxide particles (ferumoxtran-10) with a long plasma circulation time have been shown to be suitable as a MR contrast agent for intravenous MR lymphangiography. After IV injection the ferumoxtran-10 particles are taken up by macrophages are transported to the interstitial space and from there through the lymph vessels to the lymph nodes. Once within normally functioning nodes the intracellular ferumoxtran-10 within the macrophages reduces the signal intensity of normal node tissue, because of the T1- and T2*-susceptibility effect of iron oxide, thus producing a signal drop or negative enhancement. In areas of lymph nodes that are involved with malignant cells, macrophages are replaced by cancer cells. Therefore, there is in these areas no uptake of the ferumoxtran-10 particles. Using a macrophage-(= cell-) specific MR-contrast agent and high resolution MR imaging allows the detection of small and otherwise undetectable lymph node metastases in patients with cancers cancer. This has an important clinical impact, as the diagnosis will be more precise and less invasive to obtain. Subsequently this will reduce morbidity and health care costs. However, thorough knowledge of sequence parameters and planes, lymph node anatomy, appearance of normal and abnormal nodes, and pitfalls is essential when using this technique. This implies a very important role for education by expert radiologists, MR-manufacturers, and contrast agent companies.

Special session (Wed, 26 Sep, 13:30−14:30)
Recent progress in characterising sarcoma subtypes

142 INVITED

Progress in characterising sarcoma subtypes

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In the last ten years, significant improvements have been made in the classification of sarcomas. New antibodies allow to identify specific categories of sarcomas and numerous genomic abnormalities have been described: specific reciprocal translocations, gene amplifications, deletions and mutations. These DNA lesions are now daily used for subtyping sarcomas.
Immunohistochemistry plays an important role to characterise sarcomas. Numerous antibodies are now available and allow to define some sarcoma subtypes: myogenin for rhabdomyosarcomas, CKIT or CD117 for GIST, TFE3 for alveolar soft part sarcoma, INI1 for rhabdoid tumors.

Major improvements have been made in the molecular approach of sarcomas and a molecular classification of these tumors can be proposed:

- Sarcomas with a specific translocation: about 25% of soft tissue sarcomas bear a specific translocation which can be used as a diagnostic marker (see table). From a practical point of view, it is currently almost necessary to demonstrate these translocations for the diagnosis of PNET, synovial sarcoma, alveolar rhabdomyosarcoma, low grade fibromyxoid sarcoma, infantile fibrosarcoma and desmoplastic small round cell tumor given the therapeutic consequences. Translocation can be desmonstrated by RT-PCR or by FISH with commercially available break apart probes.
- Sarcomas with activating mutations: about 85% of GIST show activating mutation of either KIT or PDGFRA receptor tyrosine kinase genes. The most frequent mutation involves exon 11 of KIT followed by exon 9 of KIT and exon 18 of PDGFRA. Demonstration of these mutations are useful for the diagnosis of CD117 negative GIST, for predicting response to imatinib and to explain secondary resistance to imatinib.
- Sarcomas with inactivating mutations: malignant rhabdoid tumors show biallelic inactivation of INI1 gene with a lost of INI1 expression which can be demonstrated by immunohistochemistry. Other sarcomas, such as epithelioid sarcomas and some epithelioid malignant schwannomas show the same molecular and immunohistochemical abnormalities.
- Sarcomas with simple genomic profile showing gene amplification of a few genes. Well differentiated liposarcomas, dedifferentiated liposarcomas and intimal sarcomas show a simple genomic profile characterised by MDM2 and CDK4 amplifications associated with amplification of other genes in dedifferentiated liposarcomas. The presence of this DNA lesion can be used for differentiating a well differentiated liposarcoma-lipoma-type from a lipoma with secondary changes and for identifying dedifferentiated liposarcomas among pooly differentiated sarcomas. These amplifications can be demonstrated by immunohistochemistry, FISH or CGH-array.
- Other sarcomas usually show a complex genomic profile characterised by numerous gains and losses of genes with a frequent loss of Rb1 and alterations of P53. Leiomyosarcomas, pleomorphic rhabdomyosarcomas, pleomorphic liposarcomas, myxofibrosarcomas, poorly differentiated sarcomas (so-called MFH and fibrosarcomas) belong to this category and show no specific molecular abnormality.

In conclusion, major improvements have been made in the characterisation of sarcoma subtyping thanks to immunohistochemistry and molecular biology.

Translocations in sarcomas

Tumor	Translocation	Genes involved
PNET	t(11;22)(q24;q12)	EWS-FLI1
	t(21;22)(q22;q12)	EWS-ERG
	t(7;22)(q22;q12)	EWS-ETV1
	t(17;22)(q12;q12)	EWS-E1AF
	t(2;22)(q33;q12)	**EWS-FEV**
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	PAX3-FKHR
	t(1;13)(p36;q14)	**PAX7-FKHR**
Synovial sarcoma	t(X;18)(p11;q11)	SYT-SSX1
		SYT-SSX2
		SYT-SSX4 (rare)
Infantile fibrosarcoma	**t(12;15)(p13;q25)**	**ETV6-NTRK3**
Low grade fibromyxoid sarcoma	t(7;16)(q33;p11)	FUS-CREB3L2
	t(11;16)(p11;p11)	**FUS-CREB3L1 (rare)**
Inflammatory myofibroblastic tumor	t(1;2)(q22;p23)	TPM3-ALK
	t(2;19)(p23;p13)	TPM4-ALK
	t(2;17)(p23;q23)	CLTC-ALK
	t(2;2)(p23;q13)	**RANBP2-ALK**
Angiomatoid fibrohistiocytoma	**t(12;16)(q13;p11)**	**FUS-ATF1**
Dermatofibrosarcoma protuberans	**t(17;22)(q22;q13)**	**COL1A1-PDGFB**
Desmoplastic small round cell tumor	**t(11;22)(p13;q12)**	**EWS-WT1**
Clear cell sarcoma	**t(12;22)(q13;q12)**	**EWS-ATF1**
Alveolar soft part sarcoma	**t(X;17)(p11;q25)**	**TFE3-ASPL**
Myxoid/round cell liposarcoma	t(12;16)(q13;p11)	FUS-DDIT3
	t(12;22)(q13;q12)	**EWS-DDIT3**
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	EWS-NR4A3
	t(9;17)(q22;q11)	RBP56-NR4A3
	t(9;15)(q22;q21)	**TCF12-NR4A3**