In the past, medical research had gained enormous knowledge of relevant pathways in human carcinogenesis leading to the development of new therapeutic substances leading to an ongoing transformation of the therapeutic strategies from unspecific chemotherapy to personalized and so called precision targeting therapy (1,2). Small molecules initiated a start of a new medical era that human malignancy could come to an end. Nevertheless, the reality is quite sobering: despite the increasing possibilities to detect human cancer in early stages, due to technical possibilities in radiological imaging, the treatment success of most tumour entities lagged behind. Additionally, the overall survival rates of some tumour entities like biliary tract cancer and pancreatic cancer are still disastrous (3,4). But why didn’t the promises become true?

In vitro and in vivo investigations developed a huge number of small molecules strongly inhibiting pre-existing proteins of pathways essentially involved in hallmarks of cancer (1,2). Overcoming drug resistance mechanism (for example acquired point mutations or genetic heterogeneity with adopted alternate and compensatory pathways) limited the application of these small molecules (5). Additionally, small interfering RNA opened up a new perspective to control the post-transcriptional cellular machinery. But a major problem of those drugs is to reach adequate therapeutic concentrations at its target: the tumour cells. Therefore, bio-availability of these drugs is essentially for their pharmacological effectiveness. But how can we manage the transfer of siRNA to its target? As reviewed in detail, siRNA can be transferred in uncovered (naked) form, with chemical modifications, or in combination with viral or non-viral vectors, such as liposomes and nanoparticles (6,7). All of these possible delivery systems for siRNAs contain different advantages and disadvantages dealing with efficiency, stability, immune stimulation and toxicity (compiled in Table 1). Furthermore, another challenge is (I) how to achieve significant intra-cytoplasmic concentrations of siRNA; and (II) how to possibly monitor the efficacy of siRNA deposition. The interference of receptors, endocytosis, and trafficking of siRNA are reviewed in detail by Juliano et al. showing the complex processes from selective binding on the cell type of interest and effective endocytic internalization to release the pharmacological activity by inert endomembrane compartments (8). Our own extensive analysis with siRNA could demonstrate that (I) radioactively labelled siRNAs are quickly distributed to all organs and metabolized via the kidney and liver; (II) selective targeting of bcl-2 (B-cell lymphoma 2) or HOX-1 (heme oxygenase 1) via siRNA in vitro and in vivo is a promising approach for pancreatic or hepatocellular carcinoma; and (III) such bcl-2-specific siRNAs strategy may restore gemcitabine sensitivity in human pancreatic cancer cells (9-11).

Recently, Mahajan et al. conducted very interesting and comprising investigations dealing with siRNA silencing of polo-like kinase-1 (PLK1) in pancreatic ductal adenocarcinoma (PDAC) based on a vehicle system of superparamagnetic iron oxide nanoparticles (12).
theranostic system: a preparation that on the one hand has tumour-specific therapeutic effects and on the other can be used for monitoring the delivery and the effectiveness of the drug. This goal was achieved by conjugating siRNA with superparamagnetic iron oxide nanoparticles (SPIONS) (see US patent 5262176 or http://www.google.com/patents/US5262176). The authors choose PLK1 as target for the siRNA. PLK1 is a cell cycle component that is specifically mutated in PDAC and can be found in cell lines as well as in resected PDAC (13). Obviously, PLK1 seems to be a wonderful target in PDAC therapy, but studies revealed less specificity and high toxicity from PLK1 inhibitors (14). So the authors tried to solve the specificity problem with dextran coated SPIONS. Coating consisted of EPPT1 (tumor selective peptide) and MPAPs. EPPT1 is a non-immunogenic ligand of the PDAC specific underglycosylated mucin 1 (uMUC1) (cell surface associated) (15) and myristoylated polyarginine peptides (MPAPs) facilitates and enhances cellular uptake (16). Of particular importance is the fact that SPIONS can be detected through magnetic resonance imaging (MRI). The end product was a streptavidin (StAv) coated theranostic with five siPLK1 per nanoparticle: siPLK1-StAv-SPIONs. Specificity and effectiveness were investigated in different cancer models: a syngeneic orthotopic as well as a tumour selective endogenous LSL-KrasG12D, LSL-Trp53R172H, Pdx-1Cre model (KPC model) was treated twice a week with siPLK1-StAv-SPIONs. Tumour growth was checked with the help of a small animal MRI. All animals were divided in three groups. The first group was treated with intravenous siPLK1 only, the second group with a siControl-StAv-SPION (mismatch control siRNA), and the last group with siPLK1-StAv-SPIONs. In this way investigators were able to differentiate what would be the cause of a certain effect. At the end of treatment animals were euthanized and tissue was harvested for further investigations, such as immunoblotting, Ki67-staining in order to measure changes on cellular level. Additionally, a fluorescent live cell imaging was done. Therefore cells were conjugated with biotinylated Cy5 StAv. Cells were treated with siPLK1-StAv-SPIONs after pre-incubation with anti-MUC1 antibody and dynasore in order to proof uptake-mechanisms. Imaging was done with a transmission electron microscope (TEM). In short, investigators were able to examine specificity and effectiveness in vivo and in vitro, macroscopically and microscopically as well as changes on molecular level. With the help of TEM, Mahajan et al. could show that siPLK1 residues were not entrapped on the surface coatings and that treatment resulted in a significant increase of intracellular iron within the PDAC cell line 6606PDA. Unlike selective PLK1 inhibitors in phase I and II clinical trials (17), the authors showed a marked reduction in PLK1 expression with the result of a pronounced increase in the number of cells in the G2/M phase. Specificity and uptake investigations in a time-lapse live cell imaging showed a significant increase in MPAP(+)EPPT1(+)siPLK1-StAv-SPIONs compared to controls. That is probably owed to the fact that uMUC1 is an early and specific hallmark of PDAC (15). In addition to that, uptake was inhibited by dynasore. This underpins the authors hypothesis, that uptake is achieved by endocytosis since they stated that dynasore inhibits the clathrin-dependent endocytosis. In summary, in silico and in vitro data confirmed an increase in stability, accumulation and target specificity of siPLK1-StAv-SPIONS. Moreover, authors were able to examine and proof modes of action, explaining the uptake of siPLK1-StAv-SPIONs.

### Table 1: Characterization of known delivery systems for siRNAs according criteria of efficiency, immune stimulation, stability and toxicity [based on the reviewed data of Pan et al. (6) indicating that higher efficiency and stability is mostly paralleled by possibly more immune response reaction and cell toxicity]

<table>
<thead>
<tr>
<th>Known delivery systems for siRNAs</th>
<th>Efficiency</th>
<th>Stability</th>
<th>Immune stimulation</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked siRNA</td>
<td>↑/↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Viral</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
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<td>↑↑↑</td>
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<tr>
<td>Chemical modified siRNA</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑/↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Liposome modified siRNA</td>
<td>↑/↑↑</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑/↑↑</td>
<td>↑↑↑</td>
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Arrows indicate increase or decrease ranging from low (one arrow) to high (three arrows).
and after 6 hours of treatment) revealed decreased tumour intensity. In addition, only MPAP(+)EPPT1(+)siPLK1-StAv-SPIONs achieved the maximum uptake. After treatment for several weeks, compared to a placebo group, the siPLK1-StAv-SPIONs group showed a significant decrease of harvested tumour mass. Furthermore, immunoblotting stated an increase of caspase 9 and 3 activities resulting in an increased apoptosis activity. This is also documented by a significant increase in median survival (96 vs. 76 days). Quantitative real time polymerase chain reaction of tumour and other harvested organs showed a significant decrease in PLK1 mRNA within the tumour but no significant down-regulation of PLK1 mRNA in other organs, indicating the specificity and less cytotoxicity of siPLK1-StAv-SPIONs. Even more, PLK1 expression was only absent in tumour cells but not in the immediately adjacent tumour surrounding stroma.

For the first time, Mahajan et al. were able to design a theranostic that overcomes experimental and therapeutic limitations. PDAC is a highly malignant disease with only few and very limited options of therapy. Recent non-invasive therapy options show high toxicity and non-specificity due to the fact that nowadays remedies are not targeted. By creating a tumour specific targeted remedy, the authors initiated one next step for future anticancer therapies. siPLK1-StAv-SPIONs are convincing in manners of specificity and effectiveness as well as tolerability combined with the very useful fact of being able to monitor bioavailability and effectiveness. Mahajan et al. made their investigations in a well-considered cancer model which resembles as good as human PDAC and explains in vitro and in vivo as well as in silico changes and effects of their newly developed theranostic. The future challenge is to create similar theranostics that are targeted on other tumour entities.

Compared to the known disadvantages of the transfer systems of siRNA [see (6)], the authors of the commented manuscript mentioned that the immunogenicity of StAv must be critically considered and eventually counteracted by use of immunosuppressive drugs, whereby specific effects of the immune system (like C-reactive protein for a systemic inflammatory reaction or specific T-cell activation) are not investigated by Mahajan et al. The argument of accumulation in the body of iron oxide core nanoparticles under repetitive administration is disproved by the application of dextran-coated SPIONs in the treatment of anaemia without adverse effects (18). Nevertheless, systematic clinical investigations of adverse effects of this special carrier system combination of tumor imaging and therapy must be investigated in future.

Based on the findings of Mahajan et al. (12) the challenge of the future would be to choose and to develop specific docking molecules on the tumor target cells to improve the effectiveness of the chosen and transported anti-tumor therapeutics. Another challenge will be to visualize and to track the pharmacodynamics as well as eventually quantifying the in situ concentration of the anti cancer therapeutics (19).

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