except in one patient who experienced diarrhoea and fatigue grade 3 at 100 μg/kg. Both events were dose limiting toxicities (DLT) per protocol. Grade 3 lymphopenia has been observed within the 24 h following dosing in most of the patients dosed at ⩾30 μg/kg.
So far, 55% of the patients that have completed the first 8 weeks of treatment continued with extended treatment. The pharmacokinetic profiles of neither cetuximab nor rIL-21 seem to be affected by the combined treatment. The immune activation serum marker, soluble CD25 (sCD25), remains elevated between the weekly dosings at 30 and 100 μg/kg in a dose dependent manner.
Conclusion: The safety profile is manageable with no overlapping toxicities of concern of rIL-21 so far (3 ug/kg to 100 ug/kg) and cetuximab administered once weekly i.v.

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Toxicology and pharmacokinetics of humanized AR47A6.4.2, the first unconjugated therapeutic monoclonal antibody targeting TROP-2

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TROP-2 is a signal transduction molecule which is widely expressed in a number of human carcinomas. In the clinic, high levels of TROP-2 expression have been correlated with poor prognosis and a decrease in patient survival in human colorectal cancer as well as in head and neck carcinomas. Overexpression studies have validated Trop-2 as an oncogene, as its overexpression alone was sufficient to induce tumorigenesis in mice. The first unconjugated therapeutic antibody targeting TROP-2, AR47A6.4.2, was identified using the Arius' FunctionFIRST™ platform. The antibody demonstrated dose-dependent tumor growth inhibition in established in vivo xenograft models of pancreatic, colon, breast and prostate cancer. In vitro, studies have shown that AR47A6.4.2 treatment diminishes MAPK phosphorylation. IHC staining of human cancers demonstrated that the epitope for AR47A6.4.2 is present on the majority of human adenocarcinomas. Normal tissue binding was observed predominantly in ductal epithelium of the pancreas, liver, lung and kidney. The binding pattern was almost identical in cynomolgus monkey tissues. To further clinical development, a humanized form of the antibody was generated which had high affinity and potent anti-tumor efficacy. A dose-ranging toxicology study was carried out in cynomolgus monkeys with the humanized antibody. In the first phase, monkeys (2 per group) were given a single 1 hr infusion of 10, 25 or 80 mg/kg of huAR47A6.4.2. The animals were alert and active during the infusion and throughout the study. Clinical chemistry, coagulation, and hematology (blood cell counts or morphology) were assessed at several time-points. Upon necropsy, both of the mid-dose-treated monkeys and one of the high-dose treated monkeys had focal red spots in the large intestine that did not appear dose-dependent. A second cohort of monkeys was infused with 0, 10 and 25 mg/kg huAR47A6.4.2 every 3 days for 3 doses to confirm the findings above, and to follow up on pharmacokinetic analysis. The positive outcomes of these studies demonstrate the viability of targeting TROP-2 in a primate model at doses substantially higher than doses required for therapeutic effect in pre-clinical cancer models. These studies support the clinical development of a therapeutic monoclonal antibody targeting this novel cancer-associated target.

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Aberrant expression of glycosylation in juvenile gastrointestinal stromal tumours

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Background: Most adult gastrointestinal stromal tumors (GISTs) are thought to be caused by activating mutations in the KIT or PDGFRA gene. However, many juvenile GISTs lack either mutation and are considered to develop with a different pathogenesis. To investigate the molecular characteristics of juvenile GISTs, we analyzed the proteome difference in phosphorylated protein between adult and juvenile GISTs.
Material and Methods: Eleven GIST samples (7 adult cases and 4 juvenile cases lacking either mutation) were analyzed by using the differential display. And, the specific phophorylated proteins were digested by trypsin and searched by using LC-MS/MS. Furthermore, the analysis of post translational modification was done using the enzymes; glycopeptidase F and neuraminidase.
Results: Comparative analysis of tyrosine phosphorylated protein levels showed that juvenile GISTs possessed phosphorylated KIT in spite of lacking mutation in the KIT gene. Moreover, downstream signals of KIT were also activated as in adult GISTs. Although, SDS-PAGE gels showed that there was a difference of each KIT bands between adult and juvenile GISTs, they became the same after removal of N-glycans or sialic acids. Moreover, one of the most typical enzymes, ST6Gal1, which transfers Neu5Ac residues in α2−6 linkage to Gal β1−4GlcNAc units on N-glycans, is significantly less expressed in juvenile GISTs.
Conclusion: Aberrant expression of glycosylation in juvenile GISTs is generated by post-translational modification and may play a role in the progression of juvenile GISTs.

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HP1gamma epigenetically regulates cell differentiation and exhibits potential as a therapeutic target for various types of cancers

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Heterochromatin protein 1 (HP1) is a chromosomal protein that participates in chromatin packaging and gene silencing. Three HP1 isoforms (α, β, and γ) occur in mammals, but their functional differences are still elusive. In this study, we have found that HP1γ is decreased along with adipocyte differentiation, while HP1α and β are expressed constantly in cultured preadipocyte cells. In addition, ectopic overexpression of HP1γ inhibited adipogenesis. Furthermore, we did not detect HP1γ protein in the differentiated cells in various normal human tissues. These results suggest that the loss of HP1γ is required for cell differentiation. On the other hand, the methylation level of lysine 20 (K20) on histone H4 showed a significant correlation with HP1γ expression in these preadipocyte cells and normal tissue samples. However, all of cancer tissues examined were positive for HP1γ but sometimes negative for trimethylated histone H4 K20. Thus, dissociation of the correlation between HP1γ expression and the trimethylation may reflect the malfunction of epigenetic control. Finally, suppression of HP1γ expression restrained cell growth in various cancer-derived cell lines, suggesting that HP1γ may be an effective target for gene therapy against various human cancers. Taken together, our results have demonstrated the novel function of HP1γ in the epigenetic regulation of cell differentiation and cancer development.

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Efficient LNA-mediated antagonism of the oncogenic microRNA-155 in vitro and in vivo

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Background: microRNAs (miRNAs) are ~22 nt endogenous non-coding RNAs that post-transcriptionally repress expression of protein-coding genes by base-pairing with the 3′-untranslated regions of the target mRNAs. Emerging evidence implies that animal miRNAs play important roles in the control of many biological processes, and miRNAs are both implicated in the onset and progression of cancers. microRNA-155 (miR-155) is located within exon 2 of the non-coding B cell integration cluster (Bic) gene. Overexpression of miR-155 has been reported in haematological malignancies, such as B-cell lymphomas, and when overexpressed as a transgene in B cells, miR-155 gives rise to pre-B-cell lymphomas. Hence, miR-155 is a potential therapeutic target for treatment of haematological B-cell malignancies.
Materials and Methods: Locked nucleic acid (LNA)-modified oligonucleotides have proven outstanding in miRNA recognition and detection due to their high specificity and affinity, and recent studies have also reported efficient LNA-mediated miRNA silencing in vivo.
Results: Using the monocytic-macrophage RAW264.7 cell line as an in vitro model system, we identified a potent LNA-antimiR oligonucleotide for functional antagonism of endogenous miR-155, as demonstrated by luciferase reporter assays and by western blot analysis of miR-155 targets. Furthermore, systemic delivery by intravenous injections of the LNA-antimiR into mice resulted in miR-155 antagonism in the spleen with concomitant derepression of direct miR-155 target proteins.
Conclusion: We report here efficient LNA-mediated silencing of miR-155 in vitro and in vivo.