In conclusion, prodrugs SB 9001 and SB 9002-1 have been developed as orally bioavailable analogs of SB 9000, a novel anti-HBV agent.

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67

Characterization of Influenza Virus Clinical Isolates Obtained During Clinical Study of Arbidol

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An antiviral drug arbidol has been widely used in Russia now. Clinical trials and experience of using of this drug in the clinic have shown arbidol to be effective in preventing and treating influenzas A and B and well tolerated by patients. Our aim was to monitor the arbidol susceptibility of clinical isolates obtained in group of patients treated for influenza. Arbidol-resistant mutants were obtained by 15 passages of virus in MDCK cells in the presence of increasing from 5 to 20 μg/ml drug concentrations. Resistance of mutants was confirmed in cell ELISA and plaque activity assays and by haemolysis tests. To determine the molecular basis of arbidolresistance, the HA genes of the wild-type and arbidol-resistant mutants were sequenced. All mutants had amino acid substitutions only in the HA2 subunit, but at different positions. Paired isolates ($n = 25$) obtained from patients before and during therapy with arbidol (3×200 mg for 5 days) were studied for susceptibility to arbidol using ELISA-cell assay in MDCK cells. All isolates were equally sensitive to arbidol with IC50 falling in the range of 7.0–12.5 μg/ml and similar to IC_{50} previously observed for laboratory and clinical isolates. Two matched pairs of isolates of two patients from whom we were able to obtain days 4 and 5 samples were chosen for sequence analysis. No amino acid changes that had previously been identified in vitro as being involved with reduction of susceptibility to arbidol were observed. In our clinical study, it was shown that no arbidol resistance had emerged during 5 days of therapy of acute influenza infection.

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68

Preclinical Development of A New Class of Orally Active Drug Candidates for the Treatment of RSV Infections

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Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children under 1 year of age and is a leading cause of severe lower respiratory infections in infants and young children. It has been estimated in some U.S. communities that between 50% and 80% of bronchiolitis hospitalizations from November through April are due to RSV disease.

Prophylactic antibodies such as Synagis® (palivizumab) effectively reduce the incidence and severity of RSV disease in high-risk pediatric populations but the only antiviral treatment available for patients with RSV disease is ribavirin, a nucleoside analog with suboptimal clinical efficacy and safety profile.

We have developed a novel, potent class of small-molecule, orally available candidates that specifically target the RSV fusion glycoprotein. Representatives of this imidazoisoindolone class of fusion inhibitors are orally bioavailable in multiple species and have demonstrated efficacy in rodent models. They represent promising candidates for advancement into clinical trials for RSV.

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69

Carbohydrate-Binding Agents (CBAs) Potently Inhibit HIV Infection In Human Primary Monocytes/Macrophages and Efficiently Prevent Viral Capture and Subsequent Transmission to CD+4 T Lymphocytes

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Macrophages (M/M) are recognized as an important cellular target of HIV, and a crucial virus reservoir, producing and releasing large amounts of infectious viral particles for a long period of time. Moreover, productively infected M/M can interact with $CD4^+$ T-lymphocytes and transfer the virus to these cells. Carbohydrate-binding agents (CBAs) have been recently proposed as innovative anti-HIV compounds selectively targeting the glycans of the HIV-1 envelope glycoprotein gp120. Short pre-exposure of HIV-1 to CBAs prevents the DC-SIGN-expressing B-lymphoblast Raji cells (Raji/DC-SIGN) to efficiently bind HIV-1 and no syncytia formation occurs upon subsequent co-cultivation with CD4+ T-lymphocyte C8166 cells. Thus, the mannose-specific (i.e. the plant lectins HHA,

GNA, NPA and CA; the procaryotic cyanovirin-N (CV-N)) and the GlcNAc-specific (i.e. the plant lectin UDA) CBAs efficiently abrogate the DC-SIGN-directed HIV-1 capture and subsequent transmission to T-lymphocytes. The aim of our study is to demonstrate the ability of CBAs to inhibit HIV-1 capture in M/M, and subsequent virus transmission to $CD4^+$ T-lymphocytes. Our results show that CBAs efficiently prevent the capture of a variety of HIV-1 laboratory strains and isolates, and HIV-2 in human primary M/M cultures. Moreover, we observed that pre-exposure of HIV-1 to CBAs is able to prevent syncytia formation in co-cultures of CD4+ T-lymphocyte C8166 cells and CBA-exposed HIV-1 infected M/M. Thus CBAs can efficiently target the glycans of HIV, blocking the virus-cell interaction and preventing the transmission of the virus from M/M to $CD4^+$ T lymphocytes. The potential of CBAs to impair M/M in their capacity of capture and transmission of HIV to T-lymphocytes might be an important property to be taken into consideration in the eventual choice to select microbicide candidate drugs to the clinical setting. For these reasons, CBAs represent promising compounds able to compromise the infectivity and transmission of HIV by M/M.

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70

Generation and Characterization of Fully Human Antibodies Against Orthopoxviruses

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The genus Orthopxviruses includes several species of well-known pathogens, e.g. variola, vaccinia, cowpox and monkeypox viruses. Reemergence of monkeypox as a serious human disease in Africa have fueled renewed interest in orthopoxviruses. Vaccinia virus (VACV) was used in the past as an effective vaccine against smallpox. Although, VACV is generally safe vaccine, disseminated, life-threatening infections occur infrequently, especially in individuals with impaired immunity. Such complications can be treated by therapeutic administrations of human VACV immunoglobulin (VIG). However, their limitations include lot-to-lot variation, low content of specific antibodies and potential contamination by infectious agents. Recombinant fully human antibodies offer an obvious alternative to VIG and human antibodies from the traditional hybridomas technology.

Specific single-chain phage antibodies were selected from the synthetic phage display library of human scFvs antibodies biopanning procedure against VACV, strain Elstree. Positive clones were characterized and sequenced. One of the most promising scFv—1F4 was used for creation of fully human antibody. To generate this antibody the V genes from the 1F4 scFv were amplified by olgonucleotides specific for V genes with extensions including restriction-enzyme cleavage sites for cloning into modified pcDNA eukaryotic expression vectors carrying constant domains of human IgG1 for H-chain and L-chain correspondingly. The 293T human cells have been co-transfected with these constructs using Lipofectamine 2000 reagent. Fully human 1F4 antibody (fh 1F4) was purified from culture supernatant by affine chromatography.

Immunochemical properties of fh 1F4 obtained have been assayed by ELISA and Western-blot analysis. Specificities of the fh 1F4 were tested by ELISA using different orthopoxviruses such as VACV, cowpox virus, Ectromelia virus. Binding activity of the fh1F4 was assyed using subsequent dilutions of antigens and antibodies in ELISA, and affinity constant was calculated and compared with parental scFv. The fh 1F4 affinity constant was determined as $1.3 \times 109\,M^{-1}$, approximately 100 times more, than for the parental scFv. The fh Ab 1F4 did not neutralize vaccinia virus as a parental scFv.

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71

Susceptibility of German Porcine H3N2 Influenza A viruses Against Existing Antiviral Drugs

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Influenza A viruses (FLUAV) of subtype H3N2 are circulating in the European human population as well as in pigs. As a "mixing vessel" of avian and human FLUAV pigs may contribute to interspecies virus transmission and reassortment of viral genes including those responsible for antiviral susceptibility. During this study, the susceptibility of selected porcine H3N2 FLUAV isolated in Germany between 1982 and 1999 against: (a) the M2 ion channel blocker amantadine and (b) the neuraminidase inhibitors (NAI) oseltamivir and zanamivir was examined. Plaque reduction assay was performed to examine the amantadine phenotype. The NAI susceptibility phenotype was determined in enzyme- and cell culture-based inhibition assays. Genotypes were examined by sequencing the viral matrix protein (M), hemagglutinin (HA) and neuraminidase (NA) genes. Additionally, agglutinating properties of these viruses were compared.

In the result of antiviral studies, only two of seven isolates were shown to be amantadine-susceptible. The amino acid substitution S31N in viral M2 protein, known to confer amantadine resistance, was found in all resistant virus strains. In neuraminidase enzyme-inhibition assays all isolates were susceptible against oseltamivir and zanamivir. Both compounds inhibited virus spreading, reduced the virus yields as well as plaque size at nanomolar concentrations. But, much higher drug concentrations are necessary to achieve reduction in plaque number.

Genotyping revealed several substitutions in the NA and HA proteins including substitutions that were suggested to affect NAI susceptibility. However, neither R249K in NA nor T155Y and Q226L in HA impaired NAI susceptibility. Two isolates that