caspase-3 by a grafting approach. Fluorescence of protease sensors has significant change upon protease cleavage both in vitro and in vivo. Due to the characteristics of GFP expression without cofactors in many organs, the protease sensors will greatly contribute to the diagnosis of diseases rated to proteases activity and the tracking of kinetic process of protease activity during the diseases in vivo. Moreover, protease sensors targeted different signal peptides are useful for tracking protease activity in various cell apartments.

## MEDI 32

### Design of synthetic immunostimulatory motifs as agonists of Toll-like receptor 9: Use of $N^3$-methyl-dC and $N^1$-methyl-dG

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Toll-like receptor 9 (TLR9) recognizes synthetic and bacterial DNAs containing unmethylated CpG motifs and triggers a Th1-type immune response through a cascade of cell signaling. Through structure-activity relationship studies we identified synthetic immunostimulatory motifs and novel DNA structures that induce potent immune responses. The combination of synthetic stimulatory motifs and novel DNA structures provided immune modulatory oligonucleotides (IMOs) with distinct immune responses compared with conventional CpG DNA. In continuation to understand CpG DNA-TLR9 recognition and develop potent synthetic immunostimulatory motifs, we synthesized IMOs containing $N^3$-methyl-dC or $N^1$-methyl-dG modifications in place of C or G in CpG dinucleotide. We have studied TLR9 activation and immunostimulatory properties of the IMOs containing *N*-methyl-dC and -dG. These in vitro and in vivo studies suggest that TLR9 recognizes IMOs with $N^1$-methyl-dG in immunostimulatory motifs and induces potent immune responses.

## MEDI 33

### Antagonists of immunostimulatory CpG-DNA

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Bacterial DNA, oligodeoxynucleotides, and phosphorothioate oligodeoxynucleotides with a CpG motif are immunostimulatory. The CpG driven response is strongly inhibited by 2-arylquinolin-4-amines. Evidence has been accumulating that practical drugs for treating rheumatoid arthritis and lupus erythematosus can be found within this class of compounds. Recently, a biological receptor for the quinoline antagonists has been identified as Toll-like receptor 9 (TLR9). Unfortunately, this information is of no help in the rational design of improved antagonists because the X-ray structure

of TLR9 is not yet known. A large number of quinolines have been synthesized, assayed for their immunosuppressive activity, and analyzed by QSAR methodologies including Free-Wilson and CoMFA studies. The QSAR results have guided our efforts to find a highly active drug candidate. Several agents active at a concentration below 1 nM were designed and synthesized.

**MEDI 34**

**Site-specific ligation of antibody scFv through Cu (I) catalyzed 1,3-dipolar cycloaddition**

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A new paradigm for radionuclide pretargeted systemic radiation therapy for cancer has shown early evidence of being achievable. However, developing a modular non-immunogenic molecule that can be efficiently produced and is applicable to many targeting drugs is challenging. Antibody fragments (scFv) provide effective modules for such multi-functional, multi-valent, anti-tumor; anti-radiochelate and high affinity have been developed. In order to pretarget tumor and catch the subsequently injected chelated radiometal, multi-valent PEG-scFv conjugates need to be efficiently produced. A series of Br-PEG-azide/alkyne linkers were designed and synthesized for site-specific conjugation and ligation with scFv-cys. Although scFv and Br-PEG-alkyne/azide conjugations gave an overall efficiency of 80%, the ligation of two scFvs by Cu (I) assisted "Click" reaction yielded <5%. Presumably, these small linkers suffer intrinsically from low effective concentration and substantial steric hindrance. To overcome these "mathematical hurdles", a tri-alkyne containing linker was designed and synthesized. The Br-PEG-azide and Br-PEG-tri-alkyne linkers were first conjugated with two scFv-cys separately and "Clicked" together using copper (I) catalyzed 1,3-dipolar cycloaddition to give an excellent ligation efficiency of > 75% yield.