Case Studies Pharmaceutical Chemistry

Case Study 1: Glivec, a rationally developed, targeted anticancer drug

1. The drug Glivec is used to treat patients with chronic myolegenous leukemia. Please describe in a few words the disease (clinical course, molecular basis).

2. Which is the protein target of Glivec? Does Glivec bind to other proteins (so called off-targets)? How is the specificity of Glivec?

3. Glivec has a phenylamino core to which multiple chemical groups are linked. Please describe the roles of each chemical group.

4. Multiple clinical studies were performed with Glivec. Please summarize in a table the clinical trials by listing the number of patients, the disease state of the patients, the dose of drug, and the outcome for each clinical phase. Please indicate also the goal of each clinical trial.

5. In some patients treated with Glivec, resistances had developed. Which are the (molecular) reasons for the Glivec resistance? What could be strategies to treat Glivec-resistant patients?

6. How long did the development of Glivec take from the identification of a lead (binding to PKC) to the approval by the FDA? Please draw a timeline and indicate all important milestones.

7. Glivec is inhibiting a range of other kinases. Could the drug be used for other medical indications?

8. For the drug Glivec, several names such as STI571, imatinib, Glivec and Gleevec are used. Please explain why the different names are used.

Case Study 2: Aliskiren: the first renin inhibitor for clinical treatment

1. Please describe the RAS (renin-angiotensin system) cascade and indicate in which sites of the body the events of this cascade take place.

2. Why is there a need for a new blood pressure-lowering drug? Which are the alternative drugs and the corresponding targets?

3. Three generations of renin inhibitors had been developed. Please describe the molecules of the three generations as well as their properties.

4. How specific is the drug alikiren (vs. other aspartic proteases or renin of other species)?

5. Before the clinical trials the activities of the renin inhibitors were tested in animals. Which animal disease models were used? Which animal models are available today? What are the advantages and disadvantages of all of these models?

6. How was the clinical effect of aliskiren measured in the different clinical trials? Which questions were addressed by the clinical studies? Please try to group the various studies into phase I, II and III trials.

7. After the approval of aliskiren in 2007, Novartis had started new clinical trials. Which are the trials and why does Novartis perform additional trials?

Case Study 3: The development of COX2 inhibitors

1. Which NSAIDs were available before 1990? Please describe their chemical structures, their activities as well as their side effects.

2. What is the mechanism of action of the NSAIDs that were available before 1990? Which were the key experiments to reveal their mode of action?

3. Which were the experimental hints for the existence of a second COX isoform? How was the isoform COX2 discovered?

4. Please describe the differences between COX1 and COX2 (e.g. site of expression, function, etc.)

5. The emergence of new experimental techniques in the second half of the 20th century had facilitated the investigation of COX1 and COX2 function and the development of selective COX2 inhibitors. Please describe the new experimental techniques that were applied.

6. Several selective COX2 inhibitors were developed between 1990 and 2000. Please describe their advantages and disadvantages for patients. How did the sales of the COX2 inhibitors rofecoxib/celecoxib develop since their market entry in 1999/2000 (please try to find this information in the internet)?

7. Based on the development of COX2 inhibitors the author of the review paper concludes ‘lessons for drug discovery’. Which are the lessons and do you agree?

Case Study 4: Platensimycin: a new antibiotic targeting the biosynthesis of fatty acids

1. Why is there a need for new antibiotics? Is the market of antibiotics attractive for pharmaceutical companies?

2. How was platensimycin discovered? Please describe the experiments that have led to the identification of this molecule.

3. Please indicate the activity and selectivity of platensimycin that was determined in vitro.

4. Which effect was found for platensimycin in animal experiments? Please describe the experiments that were performed and indicate the results.

5. Please describe the experiments that had led to the identification of the protein target of platensimycin.

6. How was the structure of the complex platensimycin/protein target determined? Which information was gained by solving the structure of the molecule bound to the target?

7. Is platensimycin used as a drug in the clinic today? Please search the internet to answer this question.

Case Study 5: Repairing RNA in a genetic disorder using an antisense nucleotide

1. Which are alterations in the genetic material that cause Duchenne's muscular dystrophy?

2. Please explain the strategy of 'antisense-induced exon skipping' with the example of the antisense oligonucleotide PRO051.

3. Please describe the design of the clinical study with PRO051 (type of patients, number of patients, application route, applied molecule, quantity of applied molecule, duration).

4. Before the actual treatment, an in vitro prescreening was performed. Please describe the procedures of the prescreening and the outcome.

5. One method to determining the efficiency of the treatment was based on the measurement of the RNA in the muscle cells. Please describe the experimental procedures to quantify the RNA levels as well as the outcome of these experiments.

6. The outcome of the antisense therapy was also measured by quantifying the protein (dystrophin) levels. Please describe the experimental methods and the outcome.

7. The clinical study with PRO051 was published in December 2007. Please search the internet for further studies that have been performed in the meantime towards the development of Duchenne's muscular dystrophy therapies.

Case Study 6: Treatment of cystic fibrosis patients with an enzyme (DNase I)

1. Please describe the molecular mechanisms of the genetic disorder cystic fibrosis (mutated gene, affected protein, complications).

2. Which chemical reaction is catalyzed by the enzyme DNase I and why is it used in the treatment of patients with cystic fibrosis? Why is there a need for a recombinant protein?

3. Please describe the experimental steps that were followed to obtain the gene of human DNase I.

4. How was the DNA encoding DNase I inserted into bacterial cells for the expression of the enzyme? How was the expressed and analyzed? Please describe the experimental steps.

5. Please describe the experiments that were performed to test the activity of the recombinant enzyme.

6. Has this work led to a drug that is used in the clinic today? If so, what are the annual sales of this product?

Case Study 7: First interferon treatment of multiple sclerosis patients

1. Which is the molecular basis of multiple sclerosis (MS) and why did the authors decide to treat MS patients with interferon?

2. How many patients were enrolled in this study? Please describe the number of patients, their age and the type and stage of the disease.

3. All patients of the trial were treated with interferon. Please describe why the authors did not choose to have a placebo control group in their study.

4. How was the interferon used in the study prepared? In which form, dose and interval was it administered?

5. How was the effect of the treatment quantified? Please describe the parameters that were determined and the methods that were used.

6. What was the outcome of the study? Which were the side effects of the treatment?

7. Are interferons used today in the clinic for the treatment of multiple sclerosis patients?

Case Study 8: Humanization of antibodies

1. Why are the authors interested in 'humanizing' a rat antibody? Why were human antibodies not generated directly?

2. Which strategy did the authors propose to generate human-like antibodies?

3. The authors choose to test their 'humanization' strategy using the 'CAMPATH-1' antibody. Please describe the properties of this antibody (origin, specificity, activity), its application and how it was generated.

4. Please describe the experimental procedures that were used to graft the complementarity determining regions (CDRs) of CAMPATH-1 into a human antibody framework.

5. Which methods were used to compare the activity of the 'humanized' antibody with its parental antibody? What was the outcome of this study?

6. The 'humanized' antibody was tested in two patients. Please describe the disease state and previous treatments of these patients.

7. The 'humanized' antibody was applied to the patients by injection. Please describe the dose and the number of injections.

8. How was the effect of the treatment measured? Please describe also the outcome of the clinical trial.

Case Study 9: Engineering aggregation-resistant domain antibodies

1. What are domain antibodies (dAbs)?

2. Why do human domain antibodies tend to aggregate?

3. Which strategy did the authors propose to generate aggregation-resistant domain antibodies?

4. The authors of the article had tested their method for the generation of aggregation-resistant dAbs in model experiments. Please describe the design of the experiments and the outcome.

5. Finally, the authors had selected aggregation-resistant dAbs from a combinatorial dAb phage-library. Please describe the design of the library and the sequences of the isolated dAbs.

6. Which methods did the authors apply to analyze the aggregation propensity of the isolated dAbs? Were the isolated dAbs resistant to aggregation?

7. Are aggregation-resistant human dAbs used today as drugs?

Case Study 10: Protease-resistant peptides for oral administration

1. Why can peptides typically not be applied orally? Which properties of peptides would need to be changed to make them orally available?

2. What are double-bridged peptides? Why are they typically more stable than linear or monocyclic peptides?

3. How can double-bridged peptides be encoded by phage display?

4. Which strategy was proposed to generate protease-resistant peptides by phage display?

5. What was the format (size, shape, chemical structure) of peptide in the phage display library used?

6. How many "rounds" of phage display selection were performed? Which were the conditions in each round?

7. Were peptides isolated under high protease pressure displaying a higher proteolytic stability?

8. What was the structure of the most stable peptide isolated? Was it folded in solution?

9. Was the peptide stable in the gastrointestinal tract of mice?

10. To which second disease target was the approach applied? Outcome?

Case Study 11: DNA-encoded combinatorial macrocycle library

1. The authors have generated a combinatorial library of macrocycles. Please describe the chemical structure of the macrocycles in the library (backbone, size, building blocks).

2. For which two reasons did the authors generate macrocycles that are linked to single stranded DNA?

3. Please describe the principle of DNA-templated synthesis.

4. Please describe the experimental steps (reagents, chemical reactions, purification, etc.) that were applied to generate the combinatorial macrocycle library by DNA-templated synthesis.

5. How was the coupling of the building blocks to the growing macrocycles monitored.

6. Which building blocks were used to generate the combinatorial macrocycle library? How large was the generated macrocycle library?

7. Were macrocycle ligands to protein targets isolated with the method?

8. Had the authors published more recent work using DNA-templated synthesis and did they isolate any useful macrocycles?

Case Study 12: PROTACs

1. What is PROTAC? Which advantage does it promise over a protein inhibitor?
2. Which alternative techniques are available?
3. Previous work? What is novel in this paper?
4. Which moiety of molecules binds VHL? How was the ligand developed? Affinity?
5. Which proteins were targeted?
6. Ligands of ERRa and RIPK2? Do ligands alone degrade proteins? How were the linkers chosen?
7. How was PROTAC_ERRa tested? Did it lead to protein degradation? Did the PROTAC interfere with the natural function of VHL?
8. How was PROTAC_RIPK2 tested? Did it lead to protein degradation?
9. How was ubiquitinylation tested? Outcome? Did sub-stoichiometric catalysis happen?
10. Specificity for target protein? How was it tested?
11. In vivo experiment: What was done?