CLAIMS

1. A peptide clearing agent for clearance of a conjugate of
   a. an enzyme; and
   b. a binding molecule which binds specifically at a target location,
   from a non-target location in a subject through binding of the peptide to the active site of the enzyme, wherein the peptide clearing agent also binds to the asialoglycoprotein receptor expressed by hepatic cells to facilitate clearance through the liver.
2. The peptide of claim 1 wherein the peptide is glycosylated to facilitate clearance through the liver by binding to hepatic cells expressing an asialoglycoprotein receptor.
3. The peptide of claim 1 or 2 wherein the peptide prevents or inhibits enzyme activity upon binding to the enzyme.
4. The peptide of any preceding claim wherein the peptide is not modified by the enzyme activity.
5. The peptide of any preceding claim which comprises the dipeptide amino-naphthoic acid (ANA) – glutamate (Glu).
6. The peptide of any preceding claim which comprises the amino acid sequence serine (Ser) – Alanine (Ala) - amino-naphthoic acid (ANA) – glutamate (Glu) (SEQ ID NO: 1).
7. The peptide of claim 6 wherein the serine residue is glycosylated.
8. The peptide of any preceding claim wherein the enzyme is concentrated at a target location through specific binding of the binding molecule at the target location.
9. The peptide of any preceding claim wherein the binding molecule is an antibody or antigen-binding derivative thereof.
10. The peptide of any preceding claim wherein the enzyme acts to convert a pro-drug to an active drug at the target location.
11. The peptide of any preceding claim wherein the peptide has an affinity for the enzyme that is sufficient to permit clearance of the enzyme from the non-target location.
12. The peptide of any preceding claim wherein the peptide has a sufficiently rapid dissociation rate or high dissociation constant to not significantly affect enzyme activity at the target location.
13. The peptide of any preceding claim wherein the peptide's affinity for the enzyme is around 10 fold lower than the binding affinity of the binding molecule for its target location.
14. The peptide of any preceding claim wherein the target location is an antigen expressed by tumour cells.

15. The peptide of any of claims 2 to 14 wherein the glycosylation involves coupling of one or more galactose groups.
16. The peptide of any preceding claim wherein the peptide is no more than 10 amino acids in length.
17. The peptide of any preceding claim wherein the peptide is minimally immunogenic.
18. The peptide of any preceding claim wherein the peptide is chemically synthesized.
19. The peptide of any preceding claim wherein the enzyme is non-human in origin.
20. The peptide of any preceding claim wherein the enzyme is selected from carboxypeptidase G2, alkaline phosphatase, beta-glucoronidase, penicillin-V-amidase, beta-lactamase, beta-glucosidase and nitroreductase.
21. The peptide of claim 18 wherein the enzyme is carboxypeptidase G2 (CPG2).
22. A method of producing a peptide clearing agent as claimed in any preceding claim comprising:
    a. preparing an array of peptides, preferably based upon a starting peptide which is a structural analogue of the enzyme substrate
    b. screening the array of peptides for binding affinity for the conjugate of an enzyme and a binding molecule which binds specifically at a target location and selecting those with binding affinity
    c. optionally modifying the amino acid sequence of the peptides with binding affinity in step b and repeating step b using the modified peptides to test for an improvement in binding affinity
    d. optionally performing a substitution at each residue of the modified peptides found in step c to have improved binding affinity and repeating step b to determine if any of the substituted peptides have further improved binding affinity
    e. optionally determining whether the peptide or peptides resulting from step c or d (with improved binding affinity) have the ability to prevent or inhibit enzyme activity
    f. optionally confirming binding specificity for the enzyme by testing with a range of control proteins from the subject
    g. testing the peptide or peptides for the ability to bind to the asialoglycoprotein receptor expressed by hepatic cells to facilitate clearance through the liver,

wherein steps e and f, if performed, can be carried out in either order and step g can be carried out at any point in the method, including providing an array of peptides in step a capable of binding to the asialoglycoprotein receptor expressed by hepatic cells to facilitate clearance through the liver.

23. The method of claim 22 wherein step g comprises glycosylating the peptide or peptides and thus step a may comprise providing an array of glycosylated peptides.
24. The method of claim 22 or 23 wherein step a is performed using a peptide library.
25. The method of any of claims 22 to 24 wherein the enzyme is CPG2.
26. The method of any of claims 22 to 25 wherein the starting peptide is the tripeptide WFE.
27. The method of any of claims 22 to 26 further comprising comparing the amino acid sequence of the peptide produced by the method with a human amino acid sequence database to confirm the peptide is unlikely to have an undesired biological activity.
28. An antibody directed enzyme pro-drug therapy (ADEPT) method comprising use of a peptide of any preceding claim as a clearing agent.
29. Use of a peptide of any preceding claim as a clearing agent in antibody directed enzyme pro-drug therapy (ADEPT).
30. A peptide of any preceding claim for use as a clearing agent in antibody directed enzyme pro-drug therapy (ADEPT).
31. Use of a peptide of any preceding claim in the manufacture of a clearing agent for use in antibody directed enzyme pro-drug therapy (ADEPT).

The Claims defining the invention are as follows:

1. A peptide clearing agent for clearance of a conjugate of
    a. an enzyme; and
    b. a binding molecule which binds specifically at a target location,

    from a non-target location in a subject comprising a first domain which binds to the active site of the enzyme and a second domain which binds to the asialoglycoprotein receptor expressed by hepatic cells to facilitate clearance of the conjugate, bound via the first domain, through the liver, wherein the peptide is no more than 30 amino acids in length.
2. The peptide clearing agent of claim 1, wherein the peptide is glycosylated to facilitate clearance through the liver by binding to hepatic cells expressing an asialoglycoprotein receptor.
3. The peptide clearing agent of claim 1 or 2, wherein the peptide prevents or inhibits enzyme activity upon binding to the enzyme.
4. The peptide clearing agent of any one of the preceding claims, wherein the peptide is not modified by the enzyme activity.
5. The peptide clearing agent of any one of the preceding claims, which comprises the dipeptide amino-naphthoic acid (ANA) – glutamate (Glu).
6. The peptide clearing agent of any one of the preceding claims, which comprises the amino acid sequence serine (Ser) – Alanine (Ala) - amino-naphthoic acid (ANA) – glutamate (Glu) (SEQ ID NO: 1).
7. The peptide clearing agent of claim 6 wherein, the serine residue is glycosylated.
8. The peptide clearing agent of any one of the preceding claims, wherein the enzyme is concentrated at a target location through specific binding of the binding molecule at the target location.
9. The peptide clearing agent of any one of the preceding claims, wherein the binding molecule is an antibody or antigen-binding derivative thereof.
10. The peptide clearing agent of any one of the preceding claims, wherein the enzyme acts to convert a pro-drug to an active drug at the target location.
11. The peptide clearing agent of any one of the preceding claims, wherein the peptide has an affinity for the enzyme that is sufficient to permit clearance of the enzyme from the non-target location.
12. The peptide clearing agent of any one of the preceding claims, wherein the peptide has a sufficiently rapid dissociation rate or high dissociation constant to not significantly affect enzyme activity at the target location.

13. The peptide clearing agent of any one of the preceding claims, wherein the peptide's affinity for the enzyme is around 10 fold lower than the binding affinity of the binding molecule for its target location.

14. The peptide clearing agent of any one of the preceding claims, wherein the target location is an antigen expressed by tumour cells.

15. The peptide clearing agent of any one of claims 2 to 14, wherein the glycosylation involves coupling of one or more galactose groups.

16. The peptide clearing agent of any one of the preceding claims wherein the peptide is no more than 10 amino acids in length.

17. The peptide clearing agent of any one of the preceding claims, wherein the peptide is minimally immunogenic.

18. The peptide clearing agent of any one of the preceding claims, wherein the peptide is chemically synthesized.

19. The peptide clearing agent of any one of the preceding claims, wherein the enzyme is non-human in origin.

20. The peptide clearing agent of any one of the preceding claims, wherein the enzyme is selected from carboxypeptidase G2, alkaline phosphatase, beta-glucoronidase, penicillin-V-amidase, beta-lactamase, beta-glucosidase and nitroreductase.

21. The peptide clearing agent of claim 20, wherein the enzyme is carboxypeptidase G2 (CPG2).

22. An *in vitro* method of producing a peptide clearing agent as claimed in any one of the preceding claims, comprising:

    a. preparing an array of peptides, preferably based upon a starting peptide which is a structural analogue of the enzyme substrate

    b. screening the array of peptides for binding affinity for the conjugate of an enzyme and a binding molecule which binds specifically at a target location and selecting those with binding affinity

    c. optionally modifying the amino acid sequence of the peptides with binding affinity in step b and repeating step b using the modified peptides to test for an improvement in binding affinity

    d. optionally performing a substitution at each residue of the modified peptides found in step c to have improved binding affinity and repeating step b to determine if any of the substituted peptides have further improved binding affinity

    e. optionally determining whether the peptide or peptides resulting from step c or d have the ability to prevent or inhibit enzyme activity

f. optionally confirming binding specificity for the enzyme by testing with a range of control proteins from the subject

g. testing the peptide or peptides for the ability to bind to the asialoglycoprotein receptor expressed by hepatic cells to facilitate clearance through the liver,

wherein steps e and f, if performed, can be carried out in either order and step g can be carried out at any point in the method, including providing an array of peptides in step a capable of binding to the asialoglycoprotein receptor expressed by hepatic cells to facilitate clearance through the liver.

23. The method of claim 22, wherein step g comprises glycosylating the peptide or peptides and thus step a may comprise providing an array of glycosylated peptides.

24. The method of claim 22 or 23, wherein step a is performed using a peptide library.

25. The method of any one of claims 22 to 24, wherein the enzyme is CPG2.

26. The method of any of claims 22 to 25 wherein the starting peptide is the tripeptide Tryptophan-phenylalanine-glutamate.

27. The method of any one of claims 22 to 26, further comprising comparing the amino acid sequence of the peptide produced by the method with a human amino acid sequence database to confirm the peptide is unlikely to have an undesired biological activity.

28. A peptide clearing agent of any one of claims 1 to 21 for use as a clearing agent in antibody directed enzyme pro-drug therapy (ADEPT).

29. Use of a peptide clearing agent of any one of claims 1 to 21 in the manufacture of a clearing agent for use in antibody directed enzyme pro-drug therapy (ADEPT).