

CLAIMS

1. A variant antigen-binding domain which comprises mutations Y32F and A100N and one further mutation selected from T28K, T28R or G31R in the VH domain compared to a reference antibody having a VH domain with the sequence shown in SEQ ID NO: 1 and a VL domain with the sequence shown in SEQ ID NO: 2 and in which the variant antigen-binding domain displays an increased affinity for TRBC2 over the reference antibody.
2. A variant antigen-binding domain according to Claim 1, which comprises T28K, Y32F and A100N mutations.
3. A variant antigen-binding domain according to Claim 2, further comprising at least one mutation at a position selected from the group consisting of V2, Y27, G31, R98, Y102, N103, and A107 in the VH domain, N35 in the VL domain, and R55 in the VL domain.
4. A variant antigen-binding domain according to Claim 3, in which the at least one further mutation is selected from:
 - (a) in the VH domain:
 - V2K, V2R,
 - Y27F, Y27M, Y27N, Y27W,
 - G31K, G31R, G31S,
 - R98K,
 - Y102F, Y102L,
 - N103A, N103E, N103F, N103H, N103L, N103M, N103Q, N103S, N103W,
 - N103Y,
 - A107S,
 - and
 - (b) in the VL domain:
 - N35M, N35F, N35Y, N35K, N35R, and
 - R55K.

5. A variant antigen-binding domain according to Claim 4, which is selected from a variant antigen-binding domain comprising the following mutation combinations:

T28K, Y32F, A100N in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N in the VH domain,

5 T28K, Y32F, A100N, Y27N in the VH domain,

T28K, Y32F, A100N, G31K in the VH domain,

T28K, Y32F, A100N, Y27M in the VH domain,

T28K, Y32F, A100N, Y27W in the VH domain,

T28K, Y32F, A100N in the VH domain and R55K in the VL domain,

10 T28K, Y32F, A100N, N103H in the VH domain,

T28K, Y32F, A100N, N103A in the VH domain,

T28K, Y32F, A100N, N103Y in the VH domain,

T28K, Y32F, A100N in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, N103S in the VH domain and N35M in the VL domain,

15 T28K, Y32F, A100N, N103M in the VH domain,

T28K, Y32F, A100N, N103W in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, N103S in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, R98K in the VH domain,

20 T28K, Y32F, A100N, N103S in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, N103L in the VH domain,

T28K, Y32F, A100N, N103S in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, N103S in the VH domain and N35Y in the VL domain,

T28K, Y32F, A100N, N103L in the VH domain and N35M in the VL domain,

25 T28K, Y32F, A100N, N103L in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, N103W in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, N103L in the VH domain and N35Y in the VL domain,

T28K, Y32F, A100N, N103F in the VH domain,

T28K, Y32F, A100N, N103W in the VH domain,

T28K, Y32F, A100N, N103L in the VH domain and N35K in the VL domain,

5 T28K, Y32F, A100N, N103L in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, N103W in the VH domain and N35M in the VL domain,

T28K, Y32F, A100N, N103F in the VH domain and N35Y in the VL domain,

T28K, Y32F, A100N, Y27F in the VH domain,

T28K, Y32F, A100N, N103Q in the VH domain,

10 T28K, Y32F, A100N, N103S in the VH domain,

T28K, Y32F, A100N, N103M in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, N103F in the VH domain and N35M in the VL domain,

T28K, Y32F, A100N, N103F in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, G31R in the VH domain,

15 T28K, Y32F, A100N, N103W in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, V2R in the VH domain,

T28K, Y32F, A100N, G31S in the VH domain,

T28K, Y32F, A100N, A107S in the VH domain,

T28K, Y32F, A100N, N103E in the VH domain and N35M in the VL domain,

20 T28K, Y32F, A100N, V2K in the VH domain,

T28K, Y32F, A100N, N103E in the VH domain,

T28K, Y32F, A100N, Y102F, N103M in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, Y102F, N103M in the VH domain and N35F in the VL domain,

T28K, Y32F, A100N, Y102F, N103M in the VH domain and N35R in the VL domain,

25 T28K, Y32F, A100N, Y102F in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, N103M in the VH domain and N35M in the VL domain,

T28K, Y32F, A100N, N103M in the VH domain and N35Y in the VL domain,

T28K, Y32F, A100N, N103M in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, N103F in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, Y102L, N103W in the VH domain and N35R in the VL domain,

5 T28K, Y32F, A100N, Y102L, N103W in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, Y102F in the VH domain, and

T28K, Y32F, A100N, Y102L, N103M in the VH domain and N35R in the VL domain.

6. A variant antigen-binding domain according to Claim 5, which comprises T28K, Y32F, and A100N mutations in the VH domain and N35K mutation in the VL domain.

10 7. An antibody comprising a variant antigen-binding domain according to any preceding claim.

8. A chimeric antigen receptor (CAR) comprising a variant antigen-binding domain according to any of Claims 1 to 6, a spacer, a transmembrane domain and an endodomain.

15 9. A bispecific T-cell engager comprising a variant antigen-binding domain according to any of Claims 1 to 6 and a T-cell activation domain.

10. A nucleic acid sequence encoding a variant antigen-binding domain according to any of Claims 1 to 6, an antibody according to Claim 7, a CAR according to Claim 8, or a bispecific T-cell engager according to Claim 9.

11. A vector comprising a nucleic acid sequence according to Claim 10.

20 12. A cell which comprises a CAR according to Claim 8.

13. A method for making a cell according to Claim 12, which comprises the step of transducing or transfecting a cell with a vector according to Claim 11 which comprises a nucleic acid sequence encoding the CAR.

25 14. A conjugate comprising a variant antigen-binding domain according to any of Claims 1 to 6, or an antibody according to Claim 7, and a detectable entity or a chemotherapeutic entity.

15. A cell according to Claim 12, or an antibody according to Claim 7, or a bispecific T-cell engager according to Claim 9, or a conjugate according to Claim 14 for use in the treatment of a T-cell lymphoma or leukaemia, in which the malignant T-cells express TRBC2.

5 16. A diagnostic agent which comprises a variant antigen-binding domain according to any of Claims 1 to 6, or an antibody according to Claim 7.

17. A method for diagnosing a T-cell lymphoma or leukaemia in a subject which comprises the step of contacting a variant antigen-binding domain according to any of Claims 1 to 6, or an antibody according to Claim 7, to a sample comprising T-cells from the subject.

10 18. A method for identifying subjects with a T-cell lymphoma or leukaemia eligible for treatment with a cell according to Claim 12, or an antibody according to Claim 7, or a bispecific T-cell engager according to Claim 9, or a conjugate according to Claim 14, comprising determining the percentage of TRBC2 positive T-cells in a sample comprising T-cells from the subject.

15 19. A method for selecting a therapy comprising a cell according to Claim 12, or an antibody according to Claim 7, or a bispecific T-cell engager according to Claim 9, or a conjugate according to Claim 14 for the treatment of a subject, comprising determining the percentage of TRBC2 positive T-cells in a sample comprising T-cells from the subject.

20 20. A cell or antibody or bispecific T-cell engager or drug conjugate for use according to Claim 15, or a diagnostic agent according to Claim 16, or a method according to any of Claims 17 to 19, in which the T-cell lymphoma or leukaemia is selected from peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, T cell prolymphocytic leukaemia
25 and T-cell acute lymphoblastic leukaemia.

CLAIMS

1. A variant antigen-binding domain which comprises mutations Y32F and A100N and one further mutation selected from T28K, T28R or G31R in the VH domain having the sequence shown in SEQ ID NO: 1 and a VL domain with the sequence shown in SEQ ID NO: 2 and in which the variant antigen-binding domain displays an increased affinity for TRBC2 over a reference antibody having a VH domain with the sequence shown in SEQ ID NO: 1 and a VL domain with the sequence shown in SEQ ID NO: 2.
2. A variant antigen-binding domain according to Claim 1, which comprises T28K, Y32F and A100N mutations.
3. A variant antigen-binding domain according to Claim 2, further comprising at least one mutation at a position selected from the group consisting of V2, Y27, G31, R98, Y102, N103, and A107 in the VH domain, N35 in the VL domain, and R55 in the VL domain, in which the at least one further mutation is selected from:
 - (a) in the VH domain:
 - V2K, V2R,
 - Y27F, Y27M, Y27N, Y27W,
 - G31K, G31R, G31S,
 - R98K,
 - Y102F, Y102L,
 - N103A, N103E, N103F, N103H, N103L, N103M, N103Q, N103S, N103W, N103Y,
 - A107S,
 - and
 - (b) in the VL domain:
 - N35M, N35F, N35Y, N35K, N35R, and
 - R55K.
4. A variant antigen-binding domain according to Claim 3, which is selected from a variant antigen-binding domain comprising the following mutation combinations:

- T28K, Y32F, A100N in the VH domain and N35K in the VL domain,
T28K, Y32F, A100N in the VH domain,
T28K, Y32F, A100N, Y27N in the VH domain,
T28K, Y32F, A100N, G31K in the VH domain,
5 T28K, Y32F, A100N, Y27M in the VH domain,
T28K, Y32F, A100N, Y27W in the VH domain,
T28K, Y32F, A100N in the VH domain and R55K in the VL domain,
T28K, Y32F, A100N, N103H in the VH domain,
T28K, Y32F, A100N, N103A in the VH domain,
10 T28K, Y32F, A100N, N103Y in the VH domain,
T28K, Y32F, A100N in the VH domain and N35R in the VL domain,
T28K, Y32F, A100N, N103S in the VH domain and N35M in the VL domain,
T28K, Y32F, A100N, N103M in the VH domain,
T28K, Y32F, A100N, N103W in the VH domain and N35R in the VL domain,
15 T28K, Y32F, A100N in the VH domain and N35F in the VL domain,
T28K, Y32F, A100N, N103S in the VH domain and N35K in the VL domain,
T28K, Y32F, A100N, R98K in the VH domain,
T28K, Y32F, A100N, N103S in the VH domain and N35R in the VL domain,
T28K, Y32F, A100N, N103L in the VH domain,
20 T28K, Y32F, A100N, N103S in the VH domain and N35F in the VL domain,
T28K, Y32F, A100N, N103S in the VH domain and N35Y in the VL domain,
T28K, Y32F, A100N, N103L in the VH domain and N35M in the VL domain,
T28K, Y32F, A100N, N103L in the VH domain and N35R in the VL domain,
T28K, Y32F, A100N, N103W in the VH domain and N35K in the VL domain,
25 T28K, Y32F, A100N, N103L in the VH domain and N35Y in the VL domain,
T28K, Y32F, A100N, N103F in the VH domain,

- T28K, Y32F, A100N, N103W in the VH domain,
- T28K, Y32F, A100N, N103L in the VH domain and N35K in the VL domain,
- T28K, Y32F, A100N, N103L in the VH domain and N35F in the VL domain,
- T28K, Y32F, A100N, N103W in the VH domain and N35M in the VL domain,
- 5 T28K, Y32F, A100N, N103F in the VH domain and N35Y in the VL domain,
- T28K, Y32F, A100N, Y27F in the VH domain,
- T28K, Y32F, A100N, N103Q in the VH domain,
- T28K, Y32F, A100N, N103S in the VH domain,
- T28K, Y32F, A100N, N103M in the VH domain and N35F in the VL domain,
- 10 T28K, Y32F, A100N, N103F in the VH domain and N35M in the VL domain,
- T28K, Y32F, A100N, N103F in the VH domain and N35F in the VL domain,
- T28K, Y32F, A100N, G31R in the VH domain,
- T28K, Y32F, A100N, N103W in the VH domain and N35F in the VL domain,
- T28K, Y32F, A100N, V2R in the VH domain,
- 15 T28K, Y32F, A100N, G31S in the VH domain,
- T28K, Y32F, A100N, A107S in the VH domain,
- T28K, Y32F, A100N, N103E in the VH domain and N35M in the VL domain,
- T28K, Y32F, A100N, V2K in the VH domain,
- T28K, Y32F, A100N, N103E in the VH domain,
- 20 T28K, Y32F, A100N, Y102F, N103M in the VH domain and N35K in the VL domain,
- T28K, Y32F, A100N, Y102F, N103M in the VH domain and N35F in the VL domain,
- T28K, Y32F, A100N, Y102F, N103M in the VH domain and N35R in the VL domain,
- T28K, Y32F, A100N, Y102F in the VH domain and N35R in the VL domain,
- T28K, Y32F, A100N, N103M in the VH domain and N35M in the VL domain,
- 25 T28K, Y32F, A100N, N103M in the VH domain and N35Y in the VL domain,
- T28K, Y32F, A100N, N103M in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, N103F in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, Y102L, N103W in the VH domain and N35R in the VL domain,

T28K, Y32F, A100N, Y102L, N103W in the VH domain and N35K in the VL domain,

T28K, Y32F, A100N, Y102F in the VH domain, and

5 T28K, Y32F, A100N, Y102L, N103M in the VH domain and N35R in the VL domain.

5. A variant antigen-binding domain according to Claim 4, which comprises T28K, Y32F, and A100N mutations in the VH domain and N35K mutation in the VL domain.

6. An antibody comprising a variant antigen-binding domain according to any preceding claim.

10 7. A chimeric antigen receptor (CAR) comprising a variant antigen-binding domain according to any of Claims 1 to 5, a spacer, a transmembrane domain and an endodomain.

8. A bispecific T-cell engager comprising a variant antigen-binding domain according to any of Claims 1 to 5 and a T-cell activation domain.

9. A nucleic acid sequence encoding a variant antigen-binding domain according to any of
15 Claims 1 to 5, an antibody according to Claim 6, a CAR according to Claim 7, or a bispecific T-cell engager according to Claim 8.

10. A vector comprising a nucleic acid sequence according to Claim 9.

11. A cell which comprises a CAR according to Claim 7.

12. A method for making a cell according to Claim 11, which comprises the step of
20 transducing or transfecting a cell with a vector according to Claim 10 which comprises a nucleic acid sequence encoding the CAR.

13. A conjugate comprising a variant antigen-binding domain according to any of Claims 1 to 5, or an antibody according to Claim 6, and a detectable entity or a chemotherapeutic entity.

14. A cell according to Claim 11, or an antibody according to Claim 6, or a bispecific T-cell
25 engager according to Claim 8, or a conjugate according to Claim 13 for use in the treatment of a T-cell lymphoma or leukaemia comprising malignant T cells, in which the malignant T-cells express TRBC2.

15. A diagnostic agent for diagnosing T-cell lymphoma or leukaemia which comprises a variant antigen-binding domain according to any of Claims 1 to 5, or an antibody according to Claim 6.

16. A method for diagnosing a T-cell lymphoma or leukaemia in a subject which comprises the step of contacting a variant antigen-binding domain according to any of Claims 1 to 5, or an antibody according to Claim 6, to a sample comprising T-cells from the subject.

17. A method for identifying subjects with a T-cell lymphoma or leukaemia for treatment with a cell according to Claim 11, or an antibody according to Claim 6, or a bispecific T-cell engager according to Claim 8, or a conjugate according to Claim 13, the method comprising determining the percentage of TRBC2 positive T-cells in a sample comprising T-cells obtained from the subject, in which the subject is identified if the percentage of TRBC2 positive T-cells in the sample is high.

18. A method for selecting a therapy comprising a cell according to Claim 11, or an antibody according to Claim 6, or a bispecific T-cell engager according to Claim 8, or a conjugate according to Claim 13 for the treatment of a subject, the method comprising determining the percentage of TRBC2 positive T-cells in a sample comprising T-cells obtained from the subject, in which the therapy is selected to treat the subject if the percentage of TRBC2 positive T-cells in the sample is high.

19. A cell or antibody or bispecific T-cell engager or drug conjugate for use according to Claim 14, in which the T-cell lymphoma or leukaemia is selected from peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, T cell prolymphocytic leukaemia and T-cell acute lymphoblastic leukaemia.

20. A diagnostic agent according to Claim 15, in which the T-cell lymphoma or leukaemia is selected from peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, T cell prolymphocytic leukaemia and T-cell acute lymphoblastic leukaemia.

21. A method according to Claim 16, 17 or 18, in which the T-cell lymphoma or leukaemia is selected from peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL),
- 5 extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, T cell prolymphocytic leukaemia and T-cell acute lymphoblastic leukaemia.