Gamma-secretase inhibitors (GSIs), which are pan-Notch inhibitors that inhibit multiple Notch receptors, have been proposed for treatment of Notch-related diseases, and in fact have been used in clinical trials for the treatment of T-ALL . (See Roy et al., *Curr. Opin. Genet. Dev.* 17:52-59, 2007; Deangelo et al., *J. Clin. Oncol. 2006 ASCO Annual Meeting Proceedings Part I* 24:6586, 2006.) However, GSIs cause weight loss and intestinal goblet cell metaplasia, reflecting the role that Notch plays in determining cell fate by maintaining proliferation of intestinal crypt progenitor cells and prohibiting differentiation to a secretory cell fate. (See van Es *et al., Nature* 435:959-963, 2005). Although these side effects of pan-Notch inhibition may be manageable in a clinical setting, inhibitors that target individual Notch receptors, and therefore minimize or reduce these side effects, may be advantageous.

There is a need in the art for further therapeutic methods of treating cancer by targeting Notch receptors. The invention described herein meets the above-described needs and provides other benefits.

SUMMARY

The present ~~invention~~disclosure relates to the treatment of cancer using Notch antagonists singly or in combination. The present ~~invention~~disclosure specifically relates, in part, to the characterization of different classes of T-ALL. One class of T-ALL is sensitive to treatment with GSI and is also sensitive to treatment with a Notch1-specific antagonist. In contrast, another class of T-ALL is sensitive to treatment with GSI, but insensitive (i.e., resistant) to treatment with a Notch1-specific antagonist. As shown herein, the latter class of T-ALL is partially sensitive to treatment with a Notch3-specific antagonist, and even more sensitive to a combination of a Notch1-specific antagonist and a Notch3-specific antagonist. These results suggest a role for both Notch1 and Notch3 in leukemias, particularly T cell progenitor leukemias such as T-ALL.

In one aspect, there is provided the use of an anti-Notch3 antagonist antibody in the manufacture of a medicament for treating a Gamma-secretase inhibitor (GSI)-responsive T-cell leukemia that does not respond to a Notch1-specific antagonist.

In one embodimentaspect, a method of treating a GSI-responsive cancer that does not respond to a Notch1-specific antagonist is provided, the method comprising administering to a patient having such cancer an effective amount of a Notch3-specific antagonist. In certain embodiments, the cancer is T-cell leukemia. In certain embodiments, the T-cell leukemia is a lymphoblastic leukemia. In certain embodiments, the T-cell leukemia is T-ALL. In certain embodiments, the Notch3-specific antagonist is an anti-Notch3 antagonist antibody. In certain embodiments, the anti-Notch3 antagonist antibody is an anti-Notch3 NRR antibody. In certain embodiments, the anti-Notch3 NRR antibody binds to the LNR-A and HD-C

domains of Notch3 NRR. In certain embodiments, the anti-Notch3 NRR antibody comprises the heavy and light chain variable region CDRs of antibody 256A-4 or 256A-8. In certain embodiments, the anti-Notch3 NRR antibody is a humanized form of antibody 256A-4 or 256A-8. In certain embodiments, the anti-Notch3 antagonist antibody is an anti-Notch3 antibody that binds to one or more EGF-like repeats of Notch3.

In a further embodiment, the method further comprises administering an effective amount of a Notch1-specific antagonist. In certain embodiments, the Notch1-specific antagonist that is administered is an anti-Notch1 antagonist antibody. In certain embodiments, the anti-Notch1 antagonist antibody is an anti-Notch1 NRR antibody. In certain embodiments, the anti-Notch1 NRR antibody binds to the LNR-A, LNR-B, and HD-C domains of Notch1 NRR. In certain embodiments, the anti-Notch1 NRR antibody is selected from Antibody A, A-1, A-2, and A-3. In certain embodiments, the anti-Notch1 NRR antibody comprises the heavy and light chain variable region CDRs of an antibody selected from Antibody A, A-1, A-2, and A-3. In certain embodiments, the anti-Notch1 antagonist antibody is an anti-Notch1 antibody that binds to one or more EGF-like repeats of Notch1.

Also disclosed is~~In a further aspect of the invention,~~ an antibody that binds to activated Notch3 ICD~~ is provided~~. In certain embodiments, the antibody binds to the peptide of SEQ ID NO:4. In certain embodiments, the antibody is polyclonal. In certain embodiments, the antibody is monoclonal.

Further disclosed is~~In a further aspect of the invention,~~ a method of identifying a cancer that is suitable for treatment with an antagonist of Notch3~~ is provided~~, the method comprising contacting a sample of the cancer with an~~the~~ antibody as defined herein~~of claim 15~~, and determining whether significantly increased levels of activated Notch3 are present in the sample, wherein the presence of significantly increased levels of activated Notch3 indicates that the cancer is suitable for treatment with an antagonist of Notch3. In certain embodiments, the cancer is GSI-responsive.

The above and further aspects and embodiments of the ~~invention~~disclosure are provided herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A-1D shows an alignment of human Notch1 (SEQ ID NO:1) and mouse Notch1 (SEQ ID NO:2), with motifs and other features indicated.

Figure 2 shows the sequence of human Notch3 (SEQ ID NO:3). The EGF repeat region extends from amino acid residue 43 to 1383; the LNR modules extend from amino

WHAT IS CLAIMED IS:

1. Use of an anti-Notch3 antagonist antibody in the manufacture of a medicament for treating a Gamma-secretase inhibitor (GSI)-responsive T-cell leukemia that does not respond to a Notch1-specific antagonist.

2. The use of claim 1, wherein the T-cell leukemia is a lymphoblastic leukemia.

3. The use of claim 2, wherein the T-cell leukemia is T-lineage acute lymphoblastic leukemia (T-ALL).

4. The use of claim 1, wherein the anti-Notch3 antagonist antibody is an anti- Notch3 negative regulatory region (NRR) antibody.

5. The use of claim 4, wherein the anti-Notch3 NRR antibody binds to the first of the three LIN12/Notch Repeats (LNR-A) and extracellular amino-terminal end of Notch transmembrane subunit (HD-C) domains of Notch3 NRR.

6. The use of claim 4, wherein the anti-Notch3 NRR antibody is a humanized form of antibody 256A-4 or 256A-8, wherein:

a) the antibody 256A-4 comprises a CDR-H1 comprising the sequence GFTFSHYYMS, a CDR-H2 comprising the sequence ISNGGGRTDYPD, a CDR-H3 comprising the sequence RLDYFGGSPYFDY, a CDR-L1 comprising the sequence SASSSVSYMH, a CDR-L2 comprising the sequence EISKLAS, and a CDR-L3 comprising the sequence QQWNYPLIT; and

b) the antibody 256A-8 comprises a CDR-H1 comprising the sequence GFTFSHYYMS, a CDR-H2 comprising the sequence YINSGGGRTDYP, a CDR-H3 comprising the sequence LDYYGGSPYFDY, a CDR-L1 comprising the sequence SASSSVSYMH, a CDR-L2 comprising the sequence YEISKLAS, and a CDR-L3 comprising the sequence QQWNYPLIT.

7. The use of claim 4, wherein the anti-Notch3 NRR antibody comprises the heavy and light chain variable region Complementarity Determining Regions (CDRs) of antibody 256A-4 or 256A-8, wherein:

the CDR-H1 of the antibody 256A-4 comprises the sequence GFTFSHYYMS,
the CDR-H2 of the antibody 256A-4 comprises the sequence ISNGGGRTDYPD,
the CDR-H3 of the antibody 256A-4 comprises the sequence RLDYFGGSPYFDY,
the CDR-L1 of the antibody 256A-4 comprises the sequence SASSSVSYMH,
the CDR-L2 of the antibody 256A-4 comprises the sequence EISKLAS,
the CDR-L3 of the antibody 256A-4 comprises the sequence QQWNYPLIT,

the CDR-H1 of the antibody 256A-8 comprises the sequence GFTFSHYYMS,
the CDR-H2 of the antibody 256A-8 comprises the sequence YINSGGGRTDYP,
the CDR-H3 of the antibody 256A-8 comprises the sequence LDYYGGSPYFDY,
the CDR-L1 of the antibody 256A-8 comprises the sequence SASSSVSYMH,
the CDR-L2 of the antibody 256A-8 comprises the sequence YEISKLAS, and
the CDR-L3 of the antibody 256A-8 comprises the sequence QQWNYPLIT.

8. The use of claim 1, wherein the anti-Notch3 antagonist antibody is an anti-Notch3 antibody that binds to one or more epidermal growth factor (EGF)-like repeats of Notch3.

9. The use of claim 1, wherein the medicament further comprises an effective amount of an anti-Notch1 antagonist antibody.

10. The use of claim 9, wherein the anti-Notch1 antagonist antibody is an anti-Notch1 NRR antibody.

11. The use of claim 10, wherein the anti-Notch1 NRR antibody binds to the LNR-A, the second of the three LIN12/Notch Repeats (LNR-B), and HD-C domains of Notch1 NRR.

12. The use of claim 10, wherein the anti-Notch1 NRR antibody is selected from Antibody A, A-1, A-2, and A-3, wherein

a) Antibody A comprises a CDR-H1 comprising the sequence GFTFSSYWIH,
a CDR-H2 comprising the sequence ARINPSNGSTNYADSVKG, a CDR-H3 comprising the sequence ARGSGFRWVMDY, a CDR-L1 comprising the sequence RASQDVSTAVA, a CDR-L2 comprising the sequence SASFLYS, and a CDR-L3 comprising the sequence QQSYTTPPT;

b) Antibody A-1 comprises a CDR-H1 comprising the sequence GFTFSSYWIH,
a CDR-H2 comprising the sequence ARINPPNGSAHYADSVKG, a CDR-H3 comprising the sequence ARGSGFRWVMDY, a CDR-L1 comprising the sequence RASQDVSTAVA, a CDR-L2 comprising the sequence SASFLYS, and a CDR-L3 comprising the sequence QQSYTTPAT;

c) Antibody A-2 comprises a CDR-H1 comprising the sequence GFTFSSYWIH,
a CDR-H2 comprising the sequence ARINPPNRSNQYADSVKG, a CDR-H3 comprising the sequence ARGSGFRWVMDY, a CDR-L1 comprising the sequence RASQDVSTAVA, a CDR-L2 comprising the sequence SASFLYS, and a CDR-L3 comprising the sequence QQFYTTPST; and

d) Antibody A-3 comprises a CDR-H1 comprising the sequence GFTFSSYWIH,
a CDR-H2 comprising the sequence ARINPANGSTRYADSVKG, a CDR-H3 comprising the sequence ARGSGFRWVMDY, a CDR-L1 comprising the sequence RASQDVSTAVA, a CDR-L2 comprising the sequence SASFLYS, and a CDR-L3 comprising the sequence QQSFSTPAT.

13. The use of claim 10, wherein the anti-Notch1 NRR antibody comprises the heavy and light chain variable region CDRs of an antibody selected from Antibody A, A-1, A-2, and A-3, wherein

the CDR-H1 of Antibody A comprises the sequence GFTFSSYWIH,

the CDR-H2 of Antibody A comprises the sequence ARINPSNGSTNYADSVKG,

the CDR-H3 of Antibody A comprises the sequence ARGSGFRWVMDY,

the CDR-L1 of Antibody A comprises the sequence RASQDVSTAVA,

the CDR-L2 of Antibody A comprises the sequence SASFLYS,

the CDR-L3 of Antibody A comprises the sequence QQSYTTPPT,

the CDR-H1 of Antibody A-1 comprises the sequence GFTFSSYWIH,

the CDR-H2 of Antibody A-1 comprises the sequence ARINPPNGSAHYADSVKG,

the CDR-H3 of Antibody A-1 comprises the sequence ARGSGFRWVMDY,

the CDR-L1 of Antibody A-1 comprises the sequence RASQDVSTAVA,

the CDR-L2 of Antibody A-1 comprises the sequence SASFLYS,

the CDR-L3 of Antibody A-1 comprises the sequence QQSYTTPAT,

the CDR-H1 of Antibody A-2 comprises the sequence GFTFSSYWIH,

the CDR-H2 of Antibody A-2 comprises the sequence ARINPPNRSNQYADSVKG,

the CDR-H3 of Antibody A-2 comprises the sequence ARGSGFRWVMDY,

the CDR-L1 of Antibody A-2 comprises the sequence RASQDVSTAVA,

the CDR-L2 of Antibody A-2 comprises the sequence SASFLYS,

the CDR-L3 of Antibody A-2 comprises the sequence QQFYTTPST,

the CDR-H1 of Antibody A-3 comprises the sequence GFTFSSYWIH,

the CDR-H2 of Antibody A-3 comprises the sequence ARINPANGSTRYADSVKG,

the CDR-H3 of Antibody A-3 comprises the sequence ARGSGFRWVMDY,

the CDR-L1 of Antibody A-3 comprises the sequence RASQDVSTAVA,

the CDR-L2 of Antibody A-3 comprises the sequence SASFLYS, and

the CDR-L3 of Antibody A-3 comprises the sequence QQSFSTPAT.

14. The use of claim 9, wherein the anti-Notch1 antagonist antibody is an anti-Notch1 antibody that binds to one or more EGF-like repeats of Notch1.