CLAIMS

1) A method for targeting and processing a double-stranded DNA, comprising:

(a) Selecting one DNA target sequence of interest on one strand of a double-stranded DNA;

(b) Providing a unique compact TALEN monomer comprising:

(i) One core TALE scaffold comprising Repeat Variable Dipeptide regions (RVDs) having DNA binding specificity onto said DNA target sequence of interest;

(ii) At least one catalytic domain wherein said catalytic domain has cleavage activity on said double-stranded DNA and capable of processing DNA a few base pairs away from said DNA target sequence of interest when fused to the C and/or N terminal of said core TALE scaffold from (i);

wherein said catalytic domain comprises a meganuclease or a functional variant thereof that has at least 70%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity with the parent meganuclease,

and

wherein said catalytic domain is able to bind to the target sequence;

(iii) Optionally one peptide linker to fuse said catalytic domain from (ii) to said core TALE scaffold from (i) when needed;

wherein said compact TALEN monomer is assembled to bind and process said double stranded DNA without requiring dimerization;

(c) Contacting said double-stranded DNA with said unique monomer such that the double-stranded DNA is processed a few base pairs away in 3' and/or 5' direction(s) from said one strand target sequence.

2) A method according to claim 1, wherein said catalytic domain comprises a meganuclease variant, which is a single-chain meganuclease.

3) A method according to claim 1 or claim 2, wherein said catalytic domain comprises a meganuclease variant that comprises at least two LAGLIDADG motifs.

4) A method according to claim 3, wherein at least one of said LAGLIDADG motif is from I-CreI.

5) A method according to any one of claims 1 to 4, wherein said catalytic domain is fused to the C-terminal domain of said core TALE scaffold.

6) A method according to any one of claims 1 to 5, wherein said catalytic domain is fused to the N-terminal domain of said core TALE scaffold.

7) A method according to any one of claims 1 to 6, wherein said catalytic domain comprises a polypeptide sequence that has at least 70%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity with SEQ ID NO: 1.

8) A method according to any one of claims 1 to 7, wherein said core TALE scaffold comprises a protein sequence having at least 80%, more preferably 90%, again more preferably 95% amino acid sequence identity with the protein sequences selected from the group consisting of SEQ ID NO: 134 and SEQ ID NO: 135.

9) A method according to any one of claims 1 to 7, wherein said core TALE scaffold comprises a protein sequence having at least 80%, more preferably 90%, again more preferably 95% amino acid sequence identity with the protein sequences selected from the group consisting of SEQ ID NO: 136 to SEQ ID NO: 139.

10) A method according to any one of claims 1 to 9, wherein said compact TALEN monomer is stably or transiently expressed into cells.

11) A method according to claim 10, wherein said cells are mammalian cells.

12) A method according to claim 11, wherein said cells are primary cells.

13) A method according to claim 10 or claim 11, wherein said cells are T-cells to generate genetically engineered T cells.

14) A method according to claim 13, wherein said compact TALEN is designed to target a sequence in a T cell receptor gene.

15) A method according to claim 13 or claim 14, wherein said genetically engineered T cells are for the treatment of malignancies or infectious diseases.

16) A method according to claim 15, wherein said catalytic domain comprises a protein sequence having at least 80%, preferably 90%, more preferably 95% amino acid sequence identity with the protein sequences selected from the group of SEQ ID NO: 439-441 and SEQ ID NO: 444-446.

17) A compact TALEN monomer comprising:

(i) One core TALE scaffold comprising Repeat Variable Dipeptide regions (RVDs) having DNA binding specificity onto a specific double-stranded DNA target sequence of interest;

(ii) At least one catalytic domain comprising a meganuclease or a functional variant thereof that has at least 70%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity with the parent meganuclease, and wherein said catalytic domain is able to bind to the target sequence and has cleavage activity on said double-stranded DNA capable of processing DNA a few base pairs away from said double-stranded DNA target sequence of interest when fused to the C or N terminal of said core TALE scaffold from (i);

(iii) Optionally one peptidic linker to fuse said catalytic domain from (ii) to said engineered core TALE scaffold from (i) when needed;

wherein said compact TALEN monomer is assembled to bind said target DNA sequence and process double-stranded DNA without requiring dimerization.

18) A compact TALEN according to claim 17, wherein said catalytic domain comprises a meganuclease variant, which is a single-chain meganuclease.

19) A compact TALEN according to claim 17 or claim 18, wherein said catalytic domain comprises a meganuclease variant that comprises at least two LAGLIDADG motifs.

20) A compact TALEN according to claim 19, wherein at least one of said LAGLIDADG motif is from I-CreI.

21) A compact TALEN monomer according to any one of claims 17 to 20, wherein said catalytic domain is fused to the C-terminal domain of said core TALE scaffold.

22) A compact TALEN monomer according to any one of claims 17 to 20, wherein said catalytic domain is fused to the N-terminal domain of said core TALE scaffold.

23) A compact TALEN monomer according to any one of claims 17 to 22, wherein said catalytic domain comprises a polypeptide sequence that has at least 70%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity with (SEQ ID NO: 1).

24) A compact TALEN according to any one of claims 17 to 23, wherein said compact TALEN is designed to target a sequence in a T cell receptor gene.

25) A compact TALEN monomer according to claim 24, comprising a protein sequence having at least 80%, more preferably 90%, again more preferably 95% amino acid sequence identity with the protein sequences selected from the group of SEQ ID NO: 444-446.

26) A compact TALEN monomer according to any one of claims 17 to 25, wherein said core TALE scaffold comprises a protein sequence having at least 80%, more preferably 90%, again more preferably 95% amino acid sequence identity with the protein sequences selected from the group consisting of SEQ ID NO: 134 and SEQ ID NO: 135.

27) A compact TALEN monomer according to any one of claims 17 to 26, wherein said core TALE scaffold comprises a protein sequence having at least 80%, more preferably 90%, again more preferably 95% amino acid sequence identity with the protein sequences selected from the group consisting of SEQ ID NO: 136 to SEQ ID NO: 139.

28) A compact TALEN monomer according to any one of claims 17 to 27, wherein said peptide linker sequence can be selected from the group consisting of SEQ ID NO: 67-104 and SEQ ID NO: 372 to SEQ ID NO: 415.

29) A recombinant polynucleotide encoding a compact TALEN according to any one of claims 17 to 28.

30) A pharmaceutical composition comprising a compact TALEN according to any one of claim 17 to 28 or a recombinant polynucleotide according to claim 29.

31) Use of a compact TALEN according to any one of claims 17 to 28, a recombinant polynucleotide according to claim 29, or a pharmaceutical composition according to claim 30 in the manufacture of a medicament for the treatment of malignancies or infectious diseases.

32) A host cell which comprises a recombinant polynucleotide of claim 29.

33) A method for the in vitro or ex vivo insertion of a transgene into a specific single double-stranded DNA target sequence of a genomic locus of a cell, or tissue wherein at least one compact TALEN monomer of any one of claim 17 to 28 is introduced in said cell, or tissue.

34) A compact TALEN according to any one of claims 17 to 28, a recombinant polynucleotide according to claim 29, or a pharmaceutical composition according to claim 30 for use in therapy.