function leading to increased production of naïve lymphocytes, improved response to influenza vaccination, and extended lifespan (Chen, C et al. (2009) *Sci. Signal.* 2:ra75).

[00977] To assess the effects of mTOR inhibition on human aging-related phenotypes and whether the mTOR inhibitor RAD001 ameliorates immunosenescence, the response to influenza vaccine in elderly volunteers receiving RAD001 or placebo was evaluated. The findings presented herein suggest that RAD001 enhanced the response to influenza vaccine in elderly volunteers at doses that were well tolerated. RAD001 also reduced the percentage of programmed death (PD)-1 positive CD4 and CD8 T lymphocytes that accumulate with age. These results show that mTOR inhibition has beneficial effects on immunosenescence in elderly volunteers.

[00978] As described herein, a 6 week treatment with the mTOR inhibitor RAD001, an analog of rapamycin, improved the response to influenza vaccination in elderly human volunteers.

Methods

Study population

[00979] Elderly volunteers >= 65 years of age without unstable underlying medical diseases were enrolled at 9 sites in New Zealand and Australia. Exclusion criteria at screening included hemoglobin < 9.0 g/dL, white blood cell count <3,500/mm^3, neutrophil count <2,000/mm^3, or platelet count <125,000/mm^3, uncontrolled diabetes, unstable ischemic heart disease, clinically significant underlying pulmonary disease, history of an immunodeficiency or receiving immunosuppressive therapy, history of coagulopathy or medical condition requiring long-term anticoagulation, estimated glomerular filtration rate < 30 ml/min, presence of severe uncontrolled hypercholesterolemia (>350 mg/dL, 9.1 mmol/L) or hypertriglyceridemia (>500 mg/dL, 5.6 mmol/L).

[00980] Baseline demographics between the treatment arms were similar (Table 14). Of the 218 subjects enrolled, 211 completed the study. Seven subjects withdrew from the study. Five subjects withdrew due to adverse events (AEs), one subject withdrew consent, and one subject left the study as a result of a protocol violation.

Table 14: Demographic and Baseline characteristics of the Study Patients

Population		RAD001 0.5 mg daily N=53	RAD001 5 mg weekly N=53	RAD001 20 mg weekly N=53	Placebo pooled N=59	Total N=218
Age (Years)	Mean (SD)	70.8 (5.0)	72.0 (5.3)	71.4 (5.2)	71.1 (5.1)	71.3 (5.2)
Gender	Male– n (%)	34 (64%)	27 (51%)	32 (60%)	31 (53%)	124 (57%)
BMI* (kg/m2)	Mean (SD)	27.4 (4.2)	28.8 (5.0)	28.0 (4.1)	28.0 (4.2)	28.0 (4.4)
Race - n (%)	Caucasian	48 (91%)	50 (94%)	46 (87%)	54 (92%)	198 (91%)
	Other	5(9%)	3 (6%)	7 (13%)	5 (8%)	20 (9%)

*The body-mass index is weight in kilograms divided by the square of the height in meters.

Study Design and Conduct

[00981] From December 2011 to April 2012, 218 elderly volunteers were enrolled in a randomized, observer-blind, placebo-controlled trial. The subjects were randomized to treatment arms using a validated automated randomization system with a ratio of RAD001 to placebo of 5:2 in each treatment arm. The treatment arms were:

RAD001 0.5 mg daily or placebo

RAD001 5 mg weekly or placebo

RAD001 20 mg weekly or placebo

[00982] The trial was observer-blind because the placebo in the RAD001 0.5 mg daily and 20 mg weekly cohorts differed slightly from the RAD001 tablets in those cohorts. The study personnel evaluating the subjects did not see the study medication and therefore were fully blinded. The treatment duration for all cohorts was 6 weeks during which time subjects underwent safety evaluations in the clinic every 2 weeks. After subjects had been dosed for 4 weeks, RAD001 steady state levels were measured pre-dose and at one hour post dose. After completing the 6 week course of study drug, subjects were given a 2 week drug free break to

CLAIMS

1. Use of a composition comprising an immune effector cell, e.g., a population of immune effector cells, that expresses a CAR molecule that binds CD19, in the manufacture of a medicament wherein said medicament is to be administered sequentially or simultaneously with, one or more kinase inhibitors, for the treatment of a disease associated with expression of CD19, wherein:

(i) disease is cancer,

(ii) said one or more kinases inhibitors include a Bruton's tyrosine kinase (BTK) inhibitor, and

(iii) said immune effector cell comprises a T cell, or an NK cell.

2. The use of claim 1, wherein:

the kinase inhibitor and the CAR19-expressing cell are to be administered as a first line of therapy; or

the kinase inhibitor and the CAR19-expressing cell are to be administered in the following manner:

(i) the CAR19-expressing cell is to be administered subsequent to the cessation of the administration of the kinase inhibitor, or

(ii) the administration of the kinase inhibitor is to be begun prior to the administration of the CAR19-expressing cell, and the CAR19-expressing cell is to be administered in combination with continued administration of the kinase inhibitor.

3. The use of claim 1 or 2, wherein the disease associated with expression of CD19 is, or is identified as being, completely or partially responsive to the BTK inhibitor, e.g., ibrutinib, or completely or partially responsive to the CAR19-expressing cell.

4. The use of any of claims 1-3, wherein the BTK inhibitor is chosen from ibrutinib, GDC-0834, RN-486, CGI-560, CGI-1764, HM-71224, CC-292, ONO-4059, CNX-774, or LFM-A13.

5. The use of any of claims 2-4, wherein the kinase inhibitor is ibrutinib and the ibrutinib has a dose of about 250 mg, 300 mg, 350 mg, 400 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, or 600 mg daily.

6. The the use of any of claims 2-5, wherein the cell expresses a CAR molecule comprising an anti-CD19 binding domain, a transmembrane domain, and an intracellular signaling domain.

7. The use of claim 6, wherein the intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

8. The use of claim 6 or 7, wherein the CAR molecule comprises an anti-CD19 binding domain comprising a light chain complementarity determining region 1 (LC CDR1), a light chain complementarity determining region 2 (LC CDR2), a light chain complementarity determining region 3 (LC CDR3), a heavy chain complementarity determining region 1 (HC CDR1), a heavy chain complementarity determining region 2 (HC CDR2), and a heavy chain complementarity determining region 3 (HC CDR3) of an anti-CD19 binding domain chosen from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO: 59.

9. The use of claim 8, wherein:

(i) the anti-CD19 binding domain comprises a murine light chain variable region of SEQ ID NO: 98, SEQ ID NO: 58, or SEQ ID NO: 59, and a murine heavy chain variable region of SEQ ID NO: 98, SEQ ID NO: 58, or SEQ ID NO: 59;

(ii) the anti-CD19 binding domain comprises a LC CDR1 of SEQ ID NO: 25, a LC CDR2 of SEQ ID NO: 26, and a LC CDR3 of SEQ ID NO: 27; and
a HC CDR1 of SEQ ID NO: 19, a HC CDR2 of any of SEQ ID NOS: 20-23, and a HC CDR3 of SEQ ID NO: 24;

(iii) the anti-CD19 binding domain comprises the amino acid sequence of SEQ ID NO:59, or an amino acid sequence with 95-99% identity thereto;

(iv) the anti-CD19 binding domain is a humanized anti-CD19 binding domain;

(v) the anti-CD19 binding domain comprises an amino acid sequence chosen from: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12, or an amino acid sequence with 95-99% identity thereto; or

(vi) the anti-CD19 binding domain is a scFv that comprises a light chain variable region attached to a heavy chain variable region via a linker, e.g., wherein the linker comprises the amino acid sequence of SEQ ID NO: 53.

10. The use of any of claims 1-9, wherein the CAR molecule comprises a transmembrane domain of a protein chosen from: the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 or CD154, wherein optionally:

(i) the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 15; or

(ii) the anti-CD19 binding domain is connected to the transmembrane domain by a hinge region, e.g., wherein the hinge region comprises the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:45.

11. The use of any of claims 1-10, wherein the CAR molecule comprises a costimulatory domain, wherein the costimulatory domain comprises a functional signaling domain of a protein chosen from: OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) or 4-1BB (CD137), e.g., wherein the costimulatory domain comprises the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO:51.

12. The use of any of claims 1-11, wherein the CAR molecule comprises an intracellular signaling domain, wherein the intracellular signaling domain comprises:

(i) a functional signaling domain of 4-1BB, a functional signaling domain of CD3 zeta, or both,

(ii) the amino acid sequence of CD27, a functional signaling domain of CD3 zeta, or both;

(iii) the amino acid sequence of SEQ ID NO: 16, the amino acid sequence of SEQ ID NO:17, or both;

(iv) the amino acid sequence of SEQ ID NO:16, the amino acid sequence of SEQ ID NO:43, or both;

(v) the amino acid sequence of SEQ ID NO: 51, the amino acid sequence of SEQ ID NO:17, or both; or

(vi) the amino acid sequence of SEQ ID NO:51, the amino acid sequence of SEQ ID NO:43, or both.

13. The use of any of claims 1-12, wherein the CAR molecule further comprises a leader sequence, e.g., wherein the leader sequence comprises the amino acid sequence of SEQ ID NO: 13.

14. The use of any of claims 1-13, wherein the CAR molecule comprises the amino acid sequence of SEQ ID NO:58, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, or SEQ ID NO:42.

15. The use of any of claims 1-14, wherein an agent which inhibits an immune inhibitory molecule chosen from: PD1, PD-L1, CTLA4, TIM3, CEACAM, e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 or TGFR beta is to be further administered.

16. The use of any of claims 1-15, comprising 1-5 x 10^8 CAR-expressing cells.

17. The use of any of claims 1-16, wherein the diseases associated with expression of CD19 is a cancer, chosen from a hematological cancer or a solid tumor.

18. The use of claim 17, wherein the hematological cancer is chosen from a leukemia or lymphoma.

19. The use of claim 18, wherein the hematological cancer is chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmocytoma of bone, extraosseous plasmocytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+ large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, or unclassifiable lymphoma.

20. The use of any of claims 1-19, wherein a cytokine, wherein the cytokine is IL-7, IL-15, or IL-21, is to be further administered.

21. The use of any of claims 1-20, wherein the CAR is a regulatable CAR (RCAR), wherein optionally the RCAR comprises:

(i) an intracellular signaling member comprising an intracellular signaling domain and a first switch domain,

(ii) an antigen binding member comprising an antigen binding domain that binds CD19 and a second switch domain; and

(iii) a transmembrane domain.

22. The use of any of claims 1-21, wherein:

(i) the disease associated with expression of CD19 has, or is identified as having, a BTK mutation;

(ii) the disease associated with expression of CD19 is a hematological cancer, and wherein resistance to the kinase inhibitor, the cell that expresses a CAR molecule, or both, is delayed or decreased; or

(iii) the disease associated with expression of CD19 is a hematological cancer, and wherein remission of the hematological cancer is prolonged, or relapse of the hematological cancer is delayed.

23. The use of any of claims 1-23, wherein the CAR19-expressing cell is to be administered in combination with a second kinase inhibitor, wherein:

(i) the second kinase inhibitor is other than ibrutinib, when the disease associated with expression of CD19 is, or is identified as being, non-responsive or relapsed to ibrutinib; or

(ii) the second kinase inhibitor is chosen from one or more of GDC-0834, RN-486, CGI-560, CGI-1764, HM-71224, CC-292, ONO-4059, CNX-774, or LFM-A13, or a combination thereof.

24. The use of any of claims 1-23, wherein:

(i) the disease associated with expression of CD19 is, or is identified as being, partially responsive to the kinase inhibitor, and themedicament, alone or in combination with the BTK inhibitor, is to be administered during the period of partial response; or

(ii) the disease associated with expression of CD19 is, or is identified as being, non-responsive, progressive, or stable disease after treatment with ibrutinib, and the medicament is to be administered, alone or in combination with a second BTK inhibitor, during the period of progressive or stable disease, wherein the second kinase inhibitor is other than ibrutinib.

25. The use of any of claims 1-24, wherein the kinase inhibitor is ibrutinib and the ibrutinib is formulated for the administration for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles, e.g., wherein the cycle length is 21 or 28 days.

26. The use of any of claims 1-25, wherein the cell and the kinase inhibitor are formulated for sequential delivery.

27. The use of any of claims 1-26, wherein administration is to be performed after lymphodepletion, wherein the lymphodepletion is to comprise administration of one or more of melphalan, cytoxan, cyclophosphamide, and fludarabine.

28. The use of any of claims 1-27, wherein the composition comprises T cells and NK cells.

29. The use of any of claims 17-28, wherein the hematological cancer is a relapsed or refractory hematological malignancy.

30. The use of any of claims 6-30, wherein the anti-CD19 binding domain comprises a LC CDR1 of SEQ ID NO: 25, a LC CDR2 of SEQ ID NO: 26, a LC CDR3 of SEQ ID NO: 27, a HC CDR1 of SEQ ID NO: 19, a HC CDR2 of any of SEQ ID NOS: 20-23, and a HC CDR3 of SEQ ID NO: 24.

31. The use of any of claims 1-30, wherein the CAR19-expressing cell is to be administered at least 1, at least 2, at least 3, at least 4, or at least 6 months after the administration of the kinase inhibitor has begun.

32. The use of any of claims 1-31, which results in a reduction in PD1-expressing T cells after the administration of the kinase inhibitor.

33. Use of a composition comprising an immune effector cell, e.g., a population of immune effector cells, that expresses a CAR polypeptide that comprises an anti-CD19 binding domain, a transmembrane domain, and an intracellular signaling domain, in the manufacture of a medicament, wherein said medicament is to be administered sequentially or simultaneously with Bruton's tyrosine kinase (BTK) inhibitor, for the treatment of a human subject having a leukemia or lymphoma, and wherein said immune effector cell comprises a T cell or an NK cell.

34. The use of claim 33, wherein the BTK inhibitor is ibrutinib.

35. The use of claim 33, wherein:
(i) the CAR polypeptide has the amino acid sequence of SEQ ID NO: 58; or
(ii) the CAR polypeptide has the amino acid sequence of SEQ ID NO: 32.

36. The use of any of claims 33-35, wherein the leukemia or lymphoma is selected from BALL, ALL, DLBCL, B-cell lymphoma, Follicular Lymphoma, MCL, NHL, Hodgkin Lymphoma, CLL or SLL.

37. A composition comprising a cell that expresses a CAR molecule that binds CD19, a "CAR19-expressing cell", and one or more kinase inhibitors, wherein said one or more kinase inhibitors includes a Bruton's tyrosine kinase (BTK) inhibitor.

38. The composition of claim 37, wherein the CAR19-expressing cell and the one or more kinase inhibitors are present in a single dose form, or as two or more dose forms.

39. The use of any of claims 1-36, or the composition of any of claims 37- 38, wherein the CAR19-expressing cell is a human immune effector cell, e.g., a human T cell or a human NK cell or population of cells.

40. The use of any of claims 1-36, wherein a CDK4 inhibitor, an mTOR inhibitor, or an MNK inhibitor is to be further administered.

41. The use of claim 40, wherein:

the CDK4 inhibitor is chosen from: palbociclib, aloisine A, flavopiridol, 2-(2-chlorophenyl)-5,7-dihydroxy-8-[(3S,4R)-3-hydroxy-1-methyl-4-piperidinyl]-4-chromenone; crizotinib (PF-02341066), P276-00, RAF265, indisulam, roscovitine, dinaciclib, BMS 387032, MLN8054, AG-024322, AT7519, AZD5438, BMS908662; or ribociclib;

the mTOR inhibitor is chosen from: rapamycin, a rapamycin analog such as everolimus, temsirolimus, ridaforolimus, semapimod, AZD8055, PF04691502, SF1126, XL765, or OSI-027; or

the MNK inhibitor is chosen from: CGP052088, CGP57380, cercosporamide, ETC-1780445-2, or 4-amino-5-(4-fluoroanilino)-pyrazolo [3,4-d] pyrimidine.