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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Mannick JB, Teo G, Bernardo P, et al. Targeting the biology of ageing with mTOR inhibitors to improve immune function in older adults: phase 2b and phase 3 randomised trials. *Lancet Respir Med* 2021; **2:** e250–62.

Appendix

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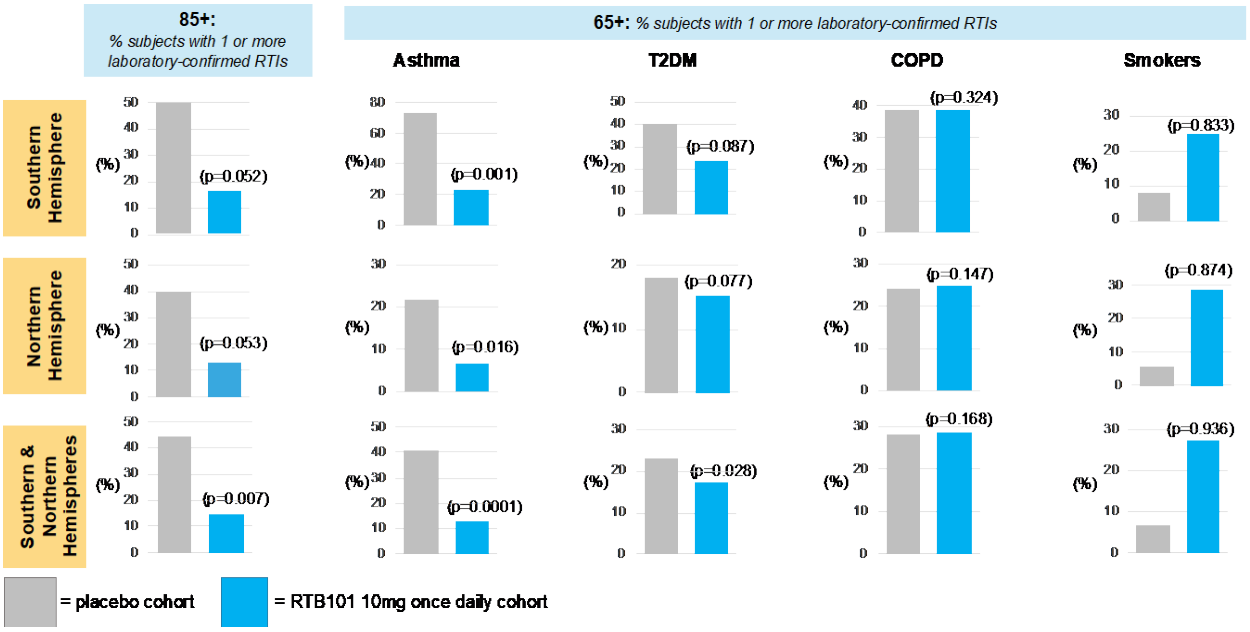

Figure S1. RTB101 did not have benefit in patients with COPD or current smokers in the Phase 2b trial. The percent of subjects in each subpopulation who developed laboratory-confirmed RTIs in the RTB101 10 mg once daily or placebo treatment group in Part 1 of the trial conducted during winter cold and flu season the southern hemisphere, Part 2 of the trial conducted during winter cold and flu season in the northern hemisphere, or in pooled subjects enrolled in both hemispheres combined is shown. The 1-sided nominal p-values for the odds ratio of having one or more laboratory-confirmed RTI in the RTB101 treatment group as compared to the placebo treatment group for each subpopulation are shown.

Table S1. Percent of subjects with symptoms that met the diagnostic criteria for respiratory tract infection/clinically symptomatic respiratory illness and the percent of subjects who had laboratory-confirmed respiratory tract infections in Phase 2b and 3 trials

	% of subjects with symptoms that met the diagnostic criteria for respiratory tract infection/clinically symptomatic respiratory illness irrespective of laboratory-confirmation of an infection	% of subjects with a laboratory-confirmed respiratory tract infection
Placebo		
Phase 2b	37.8% (68/180)	27.8% (50/180)
Phase 3	24.5% (125/510)	14.3% (73/510)
RTB101 10 mg once daily		
Phase 2b	31.8% (56/176)	19.3% (34/176)
Phase 3	26.2% (134/511)	12.7% (65/511)

Table S2. Summary of adverse events in all treatment arms in the Phase 2b trial through Week 24

Parameter (statistic)[a]	Study Part 1				Study Part 2					Pooled Study Parts 1 and 2		
	Treatment Group				Treatment Group					Treatment Group		
	BEZ 5 mg (n=61) n (%)	BEZ 10 mg QD (n=58) n (%)	Placebo (n=60) n (%)	Total (N=179) n (%)	BEZ 10 mg QD (n=118) n (%)	BEZ 10 mg BID (n=120) n (%)	BEZ + RAD QD (n=115) n (%)	Placebo (n=120) n (%)	Total (N=473) n (%)	BEZ 10 mg QD (N = 176)	Placebo (N = 180)	Total (N = 356)
Subjects with AE(s) (n [%], E)[b]	57 (93.4), 276	56 (96.6), 226	56 (93.3), 257	169 (94.4), 759	92 (78.0), 291	89 (74.2), 331	88 (76.5), 394	96 (80.0), 354	365 (77.2), 1370	148 (84.1), 517	152 (84.4), 611	300 (84.3), 1128
Subjects with TEAE(s) (n [%], E)[c]	56 (91.8), 266	56 (96.6), 216	55 (91.7), 246	167 (93.3), 728	91 (77.1), 284	88 (73.3), 317	88 (76.5), 381	94 (78.3), 337	361 (76.3), 1319	147 (83.5), 500	149 (82.8), 583	296 (83.1), 1083
Subjects with related TEAEs (n [%], E)[d]	19 (31.1), 28	18 (31.0), 26	21 (35.0), 28	58 (32.4), 82	17 (14.4), 21	22 (18.3), 36	21 (18.3), 37	18 (15.0), 42	78 (16.5), 136	35 (19.9), 47	39 (21.7), 70	74 (20.8), 117
Subjects with serious TEAEs (n [%])	4 (6.6), 9	4 (6.9), 6	7 (11.7), 15	15 (8.4), 30	4 (3.4), 4	5 (4.2), 11	11 (9.6), 13	7 (5.8), 10	27 (5.7), 38	8 (4.5), 10	14 (7.8), 25	22 (6.2), 35
Subjects with TEAEs by Severity Grade[e]												
Mild (n [%], E)	54 (88.5), 172	50 (86.2), 140	46 (76.7), 144	150 (83.8), 456	81 (68.6), 201	79 (65.8), 218	77 (67.0), 241	83 (69.2), 242	320 (67.7), 902	131 (74.4), 341	129 (71.7), 386	260 (73.0), 727
Moderate (n [%], E)	34 (55.7), 80	30 (51.7), 73	36 (60.0), 92	100 (55.9), 245	37 (31.4), 72	39 (32.5), 82	50 (43.5), 118	37 (30.8), 75	163 (34.5), 347	67 (38.1), 145	73 (40.6), 167	140 (39.3), 312
Severe (n [%], E)	12 (19.7), 14	2 (3.4), 3	6 (10.0), 10	20 (11.2), 27	8 (6.8), 9	7 (5.8), 17	10 (8.7), 22	8 (6.7), 15	33 (7.0), 63	10 (5.7), 12	14 (7.8), 25	24 (6.7), 37
Unknown severity (n [%], E)	0, 0	0, 0	0, 0	0, 0	2 (1.7), 2	0, 0	0, 0	3 (2.5), 5	5 (1.1), 7	2 (1.1), 2	3 (1.7), 5	5 (1.4), 7
Subjects with TEAEs leading to permanent study drug discontinuation (n [%], E)	4 (6.6), 5	3 (5.2), 4	5 (8.3), 5	12 (6.7), 14	6 (5.1), 7	12 (10.0), 20	5 (4.3), 16	5 (4.2), 23	28 (5.9), 66	9 (5.1), 11	10 (5.6), 28	19 (5.3), 39
Subjects with TEAEs resulting in death (n [%], E)	0, 0	0, 0	0, 0	0, 0	1 (0.8), 1	1 (0.8), 1	0, 0	1 (0.8), 1	3 (0.6), 3	1 (0.6), 1	1 (0.6), 1	2 (0.6), 2

[a] Statistic for all parameters is the number (n) (and percentage of subjects) followed by number of events (E). Denominator for percentages is the number of subjects randomized and treated in the specified treatment group.
[b] Includes events meeting AE definition from time of signing of informed consent at the Screening visit.
[c] Includes events meeting AE definition that occurred from initiation of study drug treatment.
[d] Includes treatment-emergent adverse events assessed as possibly, probably or definitely related to study drug; events with a relationship to study drug that were missing are also included.
[e] Mild defined as usually transient in nature and generally not interfering with normal activities; moderate defined as sufficiently discomforting to interfere with normal activities and severe defined as preventing normal activities.

Abbreviations: AE = adverse event; BID = twice daily; Comb. = combination; dacto. = RTB101; n = number of subjects; E = number of events; QD = once daily; TEAE = treatment-emergent adverse event

Table S3. Adverse events occurring in more than 2% of subjects treated with RTB101 10 mg once daily or placebo during 16 weeks of treatment in the Phase 2b trial

Preferred Term	Placebo (N=180) n (%)	RTB101 10 mg QD (N=176) n (%)
Headache	13 (7.2)	10 (5.7)
Constipation	10 (5.6)	3 (1.7)
Diarrhea	6 (3.3)	8 (4.5)
Fatigue	6 (3.3)	6 (3.4)
Fall	6 (3.3)	6 (3.4)
Nausea	6 (3.3)	1 (0.6)
Anemia	5 (2.8)	2 (1.1)
Arthralgia	5 (2.8)	2 (1.1)
Blood creatinine increased	4 (2.2)	4 (2.3)
Hypertension	3 (1.7)	4 (2.3)
Pain	4 (2.2)	2 (1.1)
Back Pain	2 (1.1)	4 (2.3)
Hyperglycemia	2 (1.1)	4 (2.3)
Limb injury	4 (2.2)	1 (0.6)
Tooth abscess	1 (0.6)	4 (2.3)
Decreased appetite	4 (2.2)	1 (0.6)
Skin abrasion	0	4 (2.3)

Table S4. Adverse events occurring in more than 2% of subjects treated with RTB101 10 mg once daily or placebo during 16 weeks of treatment in the Phase 3 trial

Preferred Term	Placebo (N = 510) n(%)	RTB101 10 mg QD (N = 511) n(%)
Headache	26 (5.1)	37 (7.2)
Diarrhoea	34 (6.7)	35 (6.8)
Contusion	10 (2.0)	22 (4.3)
Skin abrasion	14 (2.7)	21 (4.1)
Arthralgia	12 (2.4)	21 (4.1)
Fall	28 (5.5)	20 (3.9)
Hypertension	9 (1.8)	19 (3.7)
Asthma	12 (2.4)	18 (3.5)
Muscle strain	10 (2.0)	16 (3.1)
Urinary tract infection	21 (4.1)	15 (2.9)
Pain in extremity	8 (1.6)	13 (2.5)
Dizziness	13 (2.5)	13 (2.5)
Fatigue	11 (2.2)	12 (2.3)
Skin laceration	8 (1.6)	11 (2.2)
Back pain	15 (2.9)	11 (2.2)
Basal cell carcinoma	6 (1.2)	11 (2.2)
Oral herpes	16 (3.1)	7 (1.4)
Epistaxis	12 (2.4)	9 (1.8)
Nausea	11 (2.2)	7 (1.4)
Atrial fibrillation	10 (2.0)	6 (1.2)

Exclusion criteria for the Phase 2b trial

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1. Use of other investigational drugs within 5 half-lives of enrollment, or within 30 days, whichever is longer; or longer if required by local regulations.
2. History of hypersensitivity or allergy to RAD001, BEZ235 or their excipients or to other mTOR inhibitor drugs.
3. Subjects who require treatment with strong CYP3A4 inhibitors or inducers
4. The following cardiac conditions:
    a. Unstable angina pectoris or acute ischemic changes on ECG at screening.
    b. History of myocardial infarction, coronary bypass surgery, or any percutaneous coronary intervention (PCI) within 6 months prior to screening.
    c. Ventricular arrhythmias except for benign premature ventricular contractions
    d. Supraventricular and nodal arrhythmias not controlled with medication or a pacemaker.
    e. Symptomatic pericarditis.
    f. New York Heart Association functional classification III-IV CHF.
    g. History of familial long QT syndrome or known family history of Torsades de Pointes.
5. History of malignancy in any organ system, treated or untreated, within the past 5 years, regardless if there is evidence of local recurrence or metastases, except for the following:
    a. Localized basal cell or squamous cell carcinoma of the skin.
    b. Prostate cancer confined to the gland (AJCC stage T2N0M0 or better).
    c. Cervical carcinoma in situ.
    d. Breast cancer localized to the breast.
6. Subjects with any one of the following: hemoglobin < 10.0 g/dL for males and < 9.0 for females, white blood cell (WBC) count < 3,500/mm^3, neutrophil count < 2,000/mm^3 or platelet count < 125,000/mm^3 at screening.
7. Significant illness which (based on the participant's medical history and the clinical judgement of the investigator) has not resolved within two (2) weeks prior to initial dosing.
8. Subjects with active infection other than fungal skin or nail infection or a cold sore due to oral herpes simplex infection.
9. Recent (within the last three years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting).
10. Subjects with Type I diabetes mellitus
11. Subjects with clinically significant underlying pulmonary disease other than asthma, GOLD Class I and II COPD and chronic bronchitis.

12. Subjects with a history of a systemic autoimmune disease or receiving immunosuppressive therapy including prednisone > 10 mg for chronic use, however, short term use (i.e., ≤ 7 days of prednisone > 10 mg, e.g., to treat exacerbation of asthma, COPD, or other acute conditions) is allowed.
13. Recent surgery (involving entry into a body cavity or requiring sutures) within 2 months of the screening visit or any evidence of unhealed surgical wound or lack of significant recovery from the surgery.
    a. Minor skin surgery is allowed within 2 months of screening provided the surgical wound has healed.
14. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which may jeopardize the subject in case of participation in the study. The Investigator should make this determination in consideration of the subject's medical history and/or clinical or laboratory evidence of any of the following:
    a. Inflammatory bowel disease, major gastrointestinal tract surgery such as gastrectomy.
    b. Liver disease or liver injury as indicated by abnormal liver function tests. ALT (SGPT), AST (SGOT), alkaline phosphatase and serum bilirubin will be tested.
        i. Any **single parameter** of ALT, AST, or alkaline phosphatase must not exceed 2 x upper limit of normal (ULN) and total bilirubin/direct bilirubin must not exceed > 1.5 ULN at screening in subjects who do not have a history of Gilbert's syndrome.
            1. If the subject has a history of Gilbert's syndrome, direct and indirect reacting bilirubin should be differentiated and the direct bilirubin must be less than 1.5 times the upper limit of normal.
    c. If necessary, laboratory testing may be repeated on one occasion (as soon as possible) prior to randomization, to rule out any laboratory error.
    d. History or presence of impaired renal function as indicated by clinically significantly abnormal creatinine or BUN and/or urea values, or abnormal urinary constituents (e.g., albuminuria).
15. History of immunodeficiency diseases, including a positive HIV (ELISA and Western blot) test result.
16. Chronic infection with Hepatitis B (HBV) or Hepatitis C (HCV). A positive HBV surface antigen (HBsAg) test, or if standard local practice, a positive HBV core antigen test, excludes a subject. Subjects with a positive HCV antibody test should have HCV RNA levels measured. Subjects with positive (detectable) HCV RNA should be excluded.

17. History of drug abuse or unhealthy alcohol use within the 12 months prior to dosing, or evidence of such abuse as indicated by laboratory assays for drug and alcohol levels, or breathalyzer test for alcohol conducted during screening. (if subjects test positive for marijuana or opioid they will not be excluded if they are prescribed the drugs for a medical condition).
    a. Unhealthy alcohol use is defined as a history of, or current alcohol misuse/abuse, defined as “Five or more drinks on the same occasion on each of 5 or more days in the past 30 days”.
18. Any medical condition, as judged by the investigator, that is likely to interfere with the patient’s participation in the study, or likely to cause serious adverse events during the study.
19. Subjects with a Mini Mental Status Examination (MMSE) score < 24 at screening
20. Subjects who have received a mTOR inhibitor in the past including those subjects who received mTOR inhibitor treatment in the RAD002X2202 or CBEZ235Y2201 study.
21. Subjects with rare hereditary problems of galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption.

Exclusion criteria for the Phase 3 trial

1. Any of the following smoking criteria:
    a. Is a current smoker as assessed by medical history or a positive serum cotinine test (or positive urine cotinine test if serum cotinine testing is unavailable) at Screening.
    b. Stopped smoking ≤1 year prior to Screening.
    c. Is a previous smoker with a ≥10 pack year smoking history.
    d. Has a household member who currently smokes in the house.
2. Subjects with a medical history of clinically significant lung diseases other than asthma (e.g., chronic obstructive pulmonary disease (COPD), emphysema, interstitial pulmonary fibrosis (IPF), bronchiectasis, etc.).
3. Subjects with a Mini Mental Status Examination (MMSE) score <24 at Screening.
4. Subjects with current evidence of a serious and/or unstable medical disorder including cardiovascular, respiratory, gastrointestinal, renal (including subjects with an estimated glomerular filtration rate (eGFR) as estimated by the modified diet in renal disease (MDRD) GFR equation ≤30 mL/min/1.73m2), or hematologic disorders.
5. The following cardiac conditions:
    a. Unstable angina pectoris or acute ischemic changes on ECG at Screening or Baseline
    b. History of MI, coronary bypass surgery, or any percutaneous coronary intervention (PCI) within 6 months prior to Screening
    c. New York Heart Association functional classification III-IV congestive heart failure
    d. Unstable or life-threatening cardiac arrhythmia

        (Chronic stable atrial fibrillation is allowed)
    e. QTcF >480 msec at Screening or Baseline
6. Subjects with history of malignancy in any organ system within the past 5 years EXCEPT for the following:
    a. Localized basal cell or squamous cell carcinoma of the skin.
    b. Prostate cancer confined to the gland (AJCC stage T2N0M0 or better).
    c. Cervical carcinoma in situ.
    d. Breast cancer localized to the breast
7. Any RTI or acute significant illness (based on the subject's medical history and the clinical judgement of the Investigator) which has not resolved at least two (2) weeks prior to Baseline.

8. Subjects with a history of systemic autoimmune diseases (e.g., lupus, inflammatory bowel disease, rheumatoid arthritis, etc.), or receiving immunosuppressive therapy (such as mycophenolate, tacrolimus, cyclosporine, azathioprine, infliximab) including chronic use of prednisone >10 mg daily (however, inhaled corticosteroids and the acute use of higher doses of prednisone to treat conditions such as exacerbation of asthma or other acute conditions are allowed).
9. Subjects with Type I diabetes mellitus.
10. Clinically relevant abnormal laboratory values suggesting an unknown disease and requiring further evaluation.
11. Subjects with any one of the following during Screening:
    a. white blood cell (WBC) count <2.0 $x10^3$/microL.
    b. neutrophil count <1.0 x 10^3/microL.
    c. platelet count <75 x 10^3/microL.
12. Subjects with a history of alcohol or drug abuse within 2 years of the Screening visit.
13. Subjects with any conditions affecting absorption, distribution, or metabolism of the study drug (e.g., inflammatory bowel disease, gastric or duodenal ulcers, hepatic disease). For subjects with biochemical evidence of liver injury as indicated by abnormal liver function tests:

    • Any single parameter of ALT, AST, alkaline phosphatase or serum bilirubin must not exceed 1.5 x upper limit of normal (ULN) in subjects who do not have a history of Gilbert's syndrome. If the subject has a history of Gilbert's syndrome, direct and indirect reacting bilirubin should be differentiated, and the direct bilirubin must be less than 1.5 x ULN.

    • Any elevation above ULN of more than one parameter of ALT, AST, alkaline phosphatase or serum bilirubin will exclude a subject from participation in the study.
14. Subjects with a history of immunodeficiency diseases, including a positive human immunodeficiency virus (HIV) test result.
15. Infection with Hepatitis B (HBV) or Hepatitis C (HCV).
16. Subjects who require treatment with strong CYP3A4 or CYP1A2 inhibitors or inducers, or subjects who require treatment with digoxin.
17. Use of any other investigational medication or participation in any other investigational study within 5 half-lives of the investigational medication, or within 30 days, whichever is longer; or longer if required by local regulations.
18. Subjects who have received an organ transplant.
19. Subjects who previously received treatment with RTB101 in another clinical study (e.g., CBEZ235Y2201, RTB-BEZ235-202, or RTB-101-203).