Targeting the biology of ageing with mTOR inhibitors to improve immune function in older adults: phase 2b and phase 3 randomised trials

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Summary

Background The COVID-19 pandemic highlights the need for therapies that improve immune function in older adults, including interferon (IFN)-induced antiviral immunity that declines with age. In a previous phase 2a trial, RTB101 (previously known as BEZ235), an oral mechanistic target of rapamycin (mTOR) inhibitor, was observed to increase IFN-induced antiviral gene expression and decrease the incidence of respiratory tract infections (RTIs) in older adults. Therefore, we aimed to investigate whether oral RTB101 upregulated IFN-induced antiviral responses and decreased the incidence of viral RTIs when given once daily for 16 weeks during winter cold and flu season.

Methods We did a phase 2b and a phase 3 double-blind, randomised, placebo-controlled trial in adults aged at least 65 years enrolled in New Zealand, Australia, and the USA at 54 sites. In the phase 2b trial, patients were aged 65–85 years, with asthma, type 2 diabetes, chronic obstructive pulmonary disease (COPD), congestive heart failure, were current smokers, or had an emergency room or hospitalisation for an RTI within the past 12 months. In the phase 3 trial, patients were aged at least 65 years, did not have COPD, and were not current smokers. In the phase 2b trial, patients were randomly assigned to using a validated automated randomisation system to oral RTB101 5 mg, RTB101 10 mg once daily, or placebo in part 1 and RTB101 10 mg once daily, RTB101 10 mg twice daily, RTB101 10 mg plus everolimus once daily, or matching placebo in part 2. In the phase 3 trial, patients were randomly assigned to RTB101 10mg once daily or matching placebo. The phase 2b primary outcome was the incidence of laboratory-confirmed RTIs during 16 weeks of winter cold and influenza season and the phase 3 primary outcome was the incidence of clinically symptomatic respiratory illness defined as symptoms consistent with an RTI, irrespective of whether an infection was laboratory-confirmed. Patients, investigators, and sponsor were masked to treatment assignments. All patients who received at least part of one dose of study drug were included in the primary and safety analyses. The phase 2b trial was registered with ANZCTR, ACTRN12617000468325, ClinicalTrials.gov, NCT03373903, and the phase 3 trial was registered with ANZCTR, ACTRN12619000628145.

Findings In the phase 2b trial, we recruited 652 participants in total between May 16, 2017, and Jan 10, 2018, 179 participants to part 1 of the study (randomly assigned 1:1:1 to RTB101 5 mg once daily [61 participants], RTB101 10 mg once daily [58 participants], or matching placebo [60 participants]) and 473 patients to part 2 (randomly assigned 1:1:1:1 to RTB101 10 mg once daily [118 participants], RTB101 10 mg twice daily [120 participants], RTB101 10 mg in combination with everolimus 0·1 mg daily [115 participants] or matching placebo [120 participants]). In our first prespecified statistical analysis of the primary efficacy endpoint for part 2 of the phase 2b trial efficacy of RTB101 10 mg in combination with everolimus 0·1 mg once daily compared with placebo did not meet statistical significance but, in our second prespecified analysis, which included data from part 1 and part 2, we found a statistically significant reduction in the proportion of patients who had one or more laboratory-confirmed RTIs in the RTB101 10 mg once daily treatment group (34 [19%] of 176) compared with the pooled placebo group (50 [28%] of 180; odds ratio [OR] 0·601 [90% CI 0·391–0·922]; p=0·02). In the phase 3 trial, we enrolled 1024 patients between May 7, 2018, and July 19, 2019. 513 (50·1%) participants were randomly assigned to RTB101 10 mg once daily and 510 (49·9%) to placebo. In the full analysis set of the phase 3 trial, RTB101 did not reduce the proportion of patients with clinically symptomatic respiratory illness (134 [26%] of 511 patients in the RTB101 treatment group *vs* 125 [25%] 510 patients in the placebo treatment group; OR 1·07 [90% CI 0·80–1·42]; p=0·65). In both trials, significantly more IFN-induced antiviral genes were upregulated in patients treated with RTB101 as compared with placebo. The study drug was found to be safe and well-tolerated across trials and treatment groups. Only one patient in the placebo group in the phase 3 trial had serious adverse events (nausea, fatigue, hyponatraemia, and arthralgia) which were considered related to study drug treatment. Three patients died in the phase 2b trial and one in the phase 3 trial but no deaths were considered related to study treatment.

Interpretation The combined results indicate that low doses of the mTOR inhibitor RTB101 are well tolerated and upregulate IFN-induced antiviral responses in older adults. Further refinement of clinical trial endpoints and patient populations might be required to identify whether upregulation of IFN responses by mTOR inhibitors consistently decreases the incidence or severity of viral infections in older adults.

Introduction

Decline in the function of the ageing immune system contributes to the increased incidence of infections and the decreased response to vaccination in older adults (aged at least 65 years).[1] Respiratory tract infections (RTIs) are a leading cause of hospitalisation and deaths in older adults and are mainly caused by viruses.[2–4] Older adults might be particularly susceptible to viral RTIs due to an attenuated type 1 interferon (IFN) immune response to viruses.[5] Type 1 IFNs are the first line of defense against viruses and induce the expression of hundreds of antiviral genes that inhibit the replication of many different viruses. The type 1 IFN response might be particularly important for fighting SARS-CoV-2 infections because a substantial proportion of patients with life-threatening COVID-19 pneumonia were reported to have genetic deficiencies in the type 1 IFN pathway or neutralising autoantibodies against type 1 IFN.[6,7] In previous preclinical studies, inhibition of mechanistic target of rapamycin (mTOR) or a target downstream of mTOR protected mice from viral RTIs and upregulated IFN-induced antiviral immune responses.[8,9] Additionally, mTOR inhibition was found to increase IFN-induced antiviral gene expression and decrease the incidence of RTIs in older adults in a previous phase 2a clinical trial using RTB101 (previously known as BEZ235), an oral mTOR inhibitor.[10] Therefore, we undertook phase 2b and phase 3 clinical trials to confirm these findings and investigate whether mTOR inhibition

upregulated IFN-induced antiviral gene expression and decreased the incidence of RTIs in older adults.

Methods

Study design and participants

Phase 2b trial

We recruited participants at increased risk of RTI morbidity and mortality in a randomised, double-blind, placebo-controlled trial at ten clinical sites in New Zealand (part 1) and 17 clinical sites in the USA (part 2). Participants were aged 65–85 years with asthma, type 2 diabetes, chronic obstructive pulmonary disease (COPD), congestive heart failure, were current smokers, or had an emergency room or hospitalisation for an RTI within the past 12 months. Exclusion criteria at screening included haemoglobin less than $10·0$ g/dL for men and less than $9·0$ g/dL for women, white blood cell count less than 3500/mm³, neutrophil count less than 2000/mm³, or platelet count less than 125 000/mm³ at screening, type 1 diabetes, unstable heart disease, clinically significant underlying pulmonary disease other than asthma or COPD (Global Initiative for Chronic Obstructive Lung Diease staging criteria Class I and II), autoimmune disease, immunodeficiency or receiving immunosuppressive therapy, Mini Mental Status Examination (MMSE) score less than 24 at screening, or particpants with significant illness or infection that had not resolved within 2 weeks of screening. Complete exclusion criteria are given in the appendix (pp 8–10).

Research in context

Evidence before this study

The COVID-19 pandemic highlights the need for new therapies that enhance the function of the ageing immune system, including the type 1 interferon (IFN) immune response that declines with age but is the first line of defense against viral infections. One way to improve the function of the ageing immune system is by targeting the biological mechanisms that underlie ageing such as activity of the protein kinase mechanistic target of rapamycin (mTOR). Inhibition of mTOR has been shown to extend lifespan and improve the function of multiple ageing organ systems including the immune system in animal models. In a previous phase 2a clinical trial, RTB101, an oral mTOR inhibitor, was observed to increase type 1 IFN-induced antiviral gene expression and decrease the incidence of respiratory tract infections in older adults.

Added value of this study

The results of the phase 2b and phase 3 trials done in over 1500 adults aged at least 65 years confirm the results of the previous phase 2a trial that mTOR inhibition with RTB101 was well tolerated and consistently upregulated IFN-induced

antiviral gene expression in older adults. The upregulation of antiviral gene expression was associated with a decrease in the incidence of laboratory-confirmed RTIs in the phase 2b study but not associated with a decrease in the incidence of clinically symptomatic respiratory illness in the phase 3 trial.

Implications of all the available evidence

Despite the negative phase 3 results, important lessons were learned from this clinical development programme that is the largest to date targeting ageing biology in humans. First, the results show that it is possible to target mechanisms underlying ageing biology safely with therapies such as mTOR inhibitors in older adults. Second, the results suggest that therapies that target ageing biology in older adults might ameliorate at least some aspects of ageing organ system dysfunction (such as deficient IFN-induced antiviral responses). Further refinement of clinical endpoints and more precise identification of responder patient populations will be important in future trials of therapies targeting ageing biology to improve function in older adults.

Participants were recruited from the community and provided written informed consent.

Ethics approval was provided by the Northern B Health and Disability Ethics Committee in New Zealand and by Advarra in the USA.

Phase 3 trial

In this randomised, double-blind, placebo-controlled trial, we recruited participants aged at least 65 years who did not have COPD and were not current smokers at 16 clinical sites in New Zealand and 15 clinical sites in Australia. The change in participant enrollment criteria between the phase 2b and phase 3 trials was recommended by the US Food and Drug Administration (FDA). Exclusion criteria at screening included current smokers; COPD or other clinically significant lung diseases other than asthma; MMSE score lower than 24; current evidence of an unstable cardiac condition or other serious or unstable medical disorder including respiratory, gastrointestinal, hepatic, renal or haematological disorder; history of systemic autoimmune disease; type 1 diabetes; history of an immunodeficiency or receiving immunosuppressive therapy; white blood cell count less than $2 \cdot 0 \times 10^3/\mu L$; neutrophil count less than $1 \cdot 0 \times 10^3/\mu L$; and platelet count less than $75 \times 10^3/\mu L$. Complete exclusion criteria are given in the appendix (pp 11–12). Clinical frailty was assessed at the baseline visit using a 7-point clinical frailty scale. Randomisation was stratified according to whether a participant's clinical frailty score was 4 or more at baseline. Frailty score was not used as an exclusion criterion. Participants were recruited from the community and provided written informed consent. Ethics approval was provided by the Northern A Health and Disability Ethics Committee in New Zealand and the Bellberry Human Research Ethics Committee in Australia.

Randomisation and masking

Phase 2b trial

In part 1 of the study, participants were randomly assigned (1:1:1) to RTB101 5 mg, RTB101 10 mg once daily, or placebo using a validated automated randomisation system (Endpoint Clinical Interactive Response Technology). In part 2, participants were randomly assigned (1:1:1:1) to RTB101 10 mg once daily, RTB101 10 mg twice daily, RTB101 10 mg plus everolimus once daily, or matching placebo. All eligible participants were randomly assigned via interactive response technology (IRT) to one of the treatment groups. The IRT assigned a randomisation number to each eligible participant, which was linked to a treatment group and specified a unique medication number for the first package of study drug. Randomisation was stratified by participant age and medical histories (asthma, COPD, diabetes, current smoker, congestive heart failure, and emergency room or hospital for an RTI within the past 12 months) to prevent imbalance in treatment assignments. Masking was achieved by the use of placebo

that was identical in packaging, labelling, schedule of administration, appearance, and odour to the active drug. With the exception of the members of the data monitoring committee who did the interim analysis, participants, those giving the interventions, those assessing the outcomes, and those analysing the data were masked until final database lock.

Phase 3 trial

As in the phase 2b trial, participants were randomly assigned to RTB101 or placebo treatment groups using a validated automated randomisation system at a 1:1 ratio. Randomisation was stratified by age 85 years and older, age 65 years and older, and age younger than 85 years with a medical history of asthma, and clinical frailty scale score at least 4 to prevent imbalance in treatment assignments. Masking was achieved by the use of placebo that was identical in packaging, labelling, schedule of administration, appearance and odour to the active drug. Participants, investigators, site staff, and sponsor were masked until final database lock.

Procedures

Phase 2b trial

In part 1 of the study done during winter cold and influenza season in the southern hemisphere, eligible participants were given oral RTB101 5 mg once daily, oral RTB101 10 mg once daily, or matching placebo once daily. At the end of part 1, safety and efficacy were assessed by an unmasked data monitoring committee who chose RTB101 10 mg once daily as the dose to move to part 2 of the study. In part 2 of the study done during winter cold and flu season in the northern hemisphere, eligible participants were given oral RTB101 10 mg once daily, oral RTB101 10 mg twice daily, oral RTB101 10 mg plus RAD001 0·1 mg once daily, or matching placebo.

These dosing regimens were chosen to test whether intermittent inhibition of TORC1 predicted to be achieved with once daily RTB101 dosing was more effective than persistent inhibition predicted to be achieved with RTB101 twice daily or RTB101 in combination with everolimus once daily [11,12] for enhancing immune function in older adults.

Participants received the study drug for 16 weeks and then were followed up off the study drug for 8 weeks in both study parts. Participants underwent safety assessments, which included vital sign measurements, physical exams, and safety laboratory assessments in the clinic every 2 weeks for the first 8 weeks of the study and then every 4 weeks for the remaining 16 weeks of the study. Adverse events (including serious adverse events) were collected from the time of signing informed consent until week 24 (8 weeks after discontinuation of study drug treatment). Study drug reduction or discontinuation was allowed for participants who did not tolerate study drug treatment. RTB101 was manufactured by Aptuit (Bergamo, Italy).

RTI symptoms in participants in the trial were captured using the following methods: (1) infection diaries that were filled out by participants at home when they developed symptoms of infection (part 1 only); (2) respiratory symptom questionnaires administered by sites during twice weekly (baseline to week 16) or once weekly (Week 16–24) telephone calls with participants; (3) RTI worksheets completed by investigators when assessing participants who came to the clinic for assessment when they developed RTI symptoms; (4) Wisconsin Upper Respiratory Symptom Survey–21 completed at home by participants who developed symptoms of upper RTI; and (5) review of participants' medical record at the end of the study.

Participants were also asked to report whether RTI symptoms were mild (no limitation in daily activities), moderate (some limitation of daily activities), or severe (unable to do normal daily activities).

RTI symptoms collected using these methods were used to calculate the incidence of RTIs using predefined clinical criteria established by an expert consensus panel for surveillance of infections in older patients residing in long-term care facilities.[13] A computer program was used to assess whether respiratory symptoms reported in the case report forms met predefined clinical criteria for RTI.

Participants who developed two or more RTI symptoms were instructed to attend the clinic as soon as possible for assessment. During the clinic visit, the site staff obtained a nasopharyngeal swab for respiratory virus detection using an FDA-approved PCR panel assay (FILMARRAY respiratory panel, BioFire Diagnostics, Salt Lake City, UT, USA) that detects 17 viral and three bacterial respiratory pathogens. Additionally, laboratory confirmation was obtained by sputum Gram stain and culture in participants who developed a productive cough that represented a change from baseline and by influenza rapid antigen testing in participants who developed symptoms of influenza. To define an RTI as laboratory confirmed required at least one of these tests to be positive.

In both trials, whole blood was collected in PAXgene blood RNA tubes (BD Diagnostics, Franklin Lakes, NJ, USA) at baseline and Week 16 for RNA isolation for antiviral gene expression analysis. RNA isolation and Fluidigm gene expression was done according to good laboratory practice methods.

For the phase 2b analysis, whole-blood samples from all participants who received placebo or RTB101 10 mg once daily treatment were processed. Total RNA was isolated using the PAXgene blood RNA kit. RNA quality was assessed by spectrophotometry using a NanoDrop ND-8000 spectrophotometer (Thermofisher Scientific, Waltham, MA USA). Range-finding experiments were then carried out to identify the optimal input amounts of RNA for each TaqMan gene expression assay (Thermofisher Scientific), and RNA samples were then analysed in triplicate per assay using the 96×96 Fluidigm Dynamic GE Array (Fluidigm Corporation, San Francisco, CA, USA). Samples from the same participant were analysed on the same assay to avoid inter-batch variability. One calibrator, one no-enzyme control, and one no-template control were included with each batch of reverse transcription reactions. 20 genes known to be upregulated by IFNs and three housekeeping genes (*DECR1*, *RPLP0*, and *MAPRE2*) were measured for each sample. Each Fluidigm array was processed on Fluidigm Biomark 130, and cycle threshold (Ct) values were generated by the Fluidigm data collection software (version 4.2.1).

Phase 3 trial

Participants were treated with RTB101 10 mg or matching placebo once daily for 16 weeks during winter cold and influenza season in the southern hemisphere and then were followed up for 4 weeks off the study drug. Participants underwent safety assessments, which included vital sign measurements, physical exams, and safety laboratory assessments in the clinic every 2 weeks for the first 8 weeks of the study and then every 4 weeks for the final 12 weeks of the study. Adverse events (including serious adverse events) were collected from time of start of study drug treatment until week 20 (4 weeks after discontinuation of study drug treatment). Study drug interruption was allowed for participants who did not tolerate study drug treatment.

Participants were instructed to record in an electronic diary (eDiary) each evening throughout the 20-week study period if they had experienced one or more predefined respiratory illness symptoms during the previous 24 h that were new or reflected a change from their normal baseline symptoms. The predefined symptoms included respiratory symptoms (runny nose, sneezing, stuffy nose, sore throat, hoarseness, or cough) and general symptoms (headache, feverishness or chills, loss of appetite, body aches, or lack of energy). Participants were also asked to report if the symptoms were mild, moderate, or severe in intensity. A clinically symptomatic respiratory illness was defined as the occurrence of two respiratory symptoms (runny nose and sneezing were programmatically considered one symptom) or one respiratory and one general symptom, with at least one unique respiratory symptom being reported on two or more consecutive entries in an eDiary and at least two symptoms being at least moderate in severity. Customised proprietary software was used to a to decide whether respiratory symptoms that were entered into the eDiaries met the predefined clinical criteria for clinically symptomatic respiratory illness.

Participants who reported in their eDiary at least one unique respiratory symptom on two consecutive entries in their eDiarys were instructed to attend the study site within 48 h for assessment and to obtain laboratory testing for an infection. Laboratory confirmation of an infection was obtained using

	Study part 1				Study part 2				
	RTB101 5 mg (n=61)	RTB101 10 mg once daily (n=58)	Placebo (n=60)	Overall (n=179)	RTB101 10 mg once daily (n=118)	RTB101 10 mg twice daily (n=120)	RTB101 plus everolimus (n=115)	Placebo (n=120)	Overall (n=473)
Age at randomisation, years									
Mean (SD)	74·0 (8·2)	76·5 (7·9)	74·4 (7·3)	74·9 (7·9)	73·1 (6·9)	73·0 (6·9)	73·9 (7·0)	73·2 (7·2)	73·3 (7·0)
Median (IQR)	71·0 (68·0–79·0)	76·0 (69·0–82·0)	72·0 (69·0–79·0)	72·0 (69·0–80·0)	71·0 (68·0–77·0)	71·0 (67·5–78·0)	73·0 (68·0–78·0)	71·0 (68·0–77·0)	71·0 (68·0–78·0)
Sex									
Male	33 (54%)	31 (53%)	36 (60%)	100 (56%)	52 (44%)	61 (501%)	57 (50%)	53 (44%)	223 (47%)
Female	28 (46%)	27 (47%)	24 (40%)	79 (44%)	66 (56%)	59 (49%)	58 (50%)	67 (56%)	250 (53%)
Race									
White	56 (92%)	54 (93%)	57 (95%)	167 (93%)	114 (97%)	110 (91%)	106 (92%)	109 (91%)	439 (93%)
Black or African American	0	0	0	0	4 (3%)	9 (8%)	5 (4%)	10 (8%)	28 (6%)
Native Hawaiian or other Pacific Islander	2 (3%)	2 (3%)	2 (3%)	6 (3%)	0	0	0	0	0
Other	1 (2%)	0	1 (1·7%)	2 (1%)	0	0	2 (1·7%)	1 (<1%)	3 (<1%)
Asian	2 (3%)	2 (3%)	0	4 (2%)	0	0	1 (<1%)	0	1 (<1%)
American Indian or Alaska Native	0	0	0	0	0	1 (<1%)	1 (<1%)	0	2 (<1%)
Ethnicity									
Not-Hispanic or Latino	61 (100%)	58 (100%)	60 (100%)	179 (100%)	108 (92%)	114 (95%)	101 (88%)	108 (90%)	431 (91%)
Hispanic or Latino	0	0	0	0	10 (9%)	6 (5%)	14 (12%)	12 (10%)	42 (9%)
BMI at baseline (kg/m²)									
Mean (SD)	28·4 (5·1)	30·0 (5·8)	28·6 (5·5)	29·0 (5·5)	30·9 (6·9)	31·2 (6·1)	30·1 (6·8)	30·5 (6·0)	30·7 (6·5)

Data are n (%), unless otherwise specified.

Table 1: **Phase 2b patient demographics at baseline (full analysis set)**

three methods: (1) the FILMARRAY respiratory panel PCR assay of nasopharyngeal swabs in all participants; (2) sputum Gram stain and culture obtained in participants with a productive cough that was new or a change from their normal baseline; (3) rapid influenza detection test obtained in participants with influenza-like illness symptoms. To be classified as a laboratory confirmed RTI, at least one of these tests was required to be positive.

For the phase 3 analysis of antiviral gene expression analysis, whole-blood samples from 180 participants who received placebo and 180 participants who received RTB101 10 mg once daily treatment were processed in the same way as in the phase 2 trial. These participants in each treatment group were chosen as follows: first, all participants who had at least one laboratory-confirmed RTI up to week 16 were included. Next, any participant who did not have a whole-blood sample at both baseline and week 16 was removed. Finally, participants with no laboratory-confirmed RTI up to week 16 but had a whole-blood sample at both baseline and week 16 were randomly included, to get to a total of 180 participants per treatment group. RNA isolation and Fluidigm gene expression analysis proceeded as in the phase 2b analysis. This sample size was based on the largest sample size that could be analysed with the available budget.

Outcomes

Phase 2b trial

The primary objective of the study was to investigate whether the oral mTOR inhibitor RTB101 alone or in combination with the oral mTOR inhibitor everolimus decreased the incidence of laboratory-confirmed RTIs during 16 weeks of winter cold and flu season. This outcome was centrally assessed. Secondary objectives included the proportion of older patients having one or more RTIs for 16 weeks, irrespective of laboratory confirmation, and the safety and tolerability of up to three different doses of RTB101 alone and in combination with everolimus (as assessed by reports of adverse events and serious adverse events, physical examination, and safety laboratory values). Additional secondary objectives not reported in this manuscript were to investigate whether RTB101 alone or in combination with everolimus compared with placebo decreased the rate of RTIs per person for 16 weeks or 24 weeks, decreased the proportion of patients with one or more laboratory confirmed RTIs for 24 weeks, and the pharmacokinetics of up to three different doses of RTB101 given alone and in combination with everolimus.

Phase 3 trial

The US FDA requested a change in primary endpoint between the Phase 2b and 3 trials because of concerns that
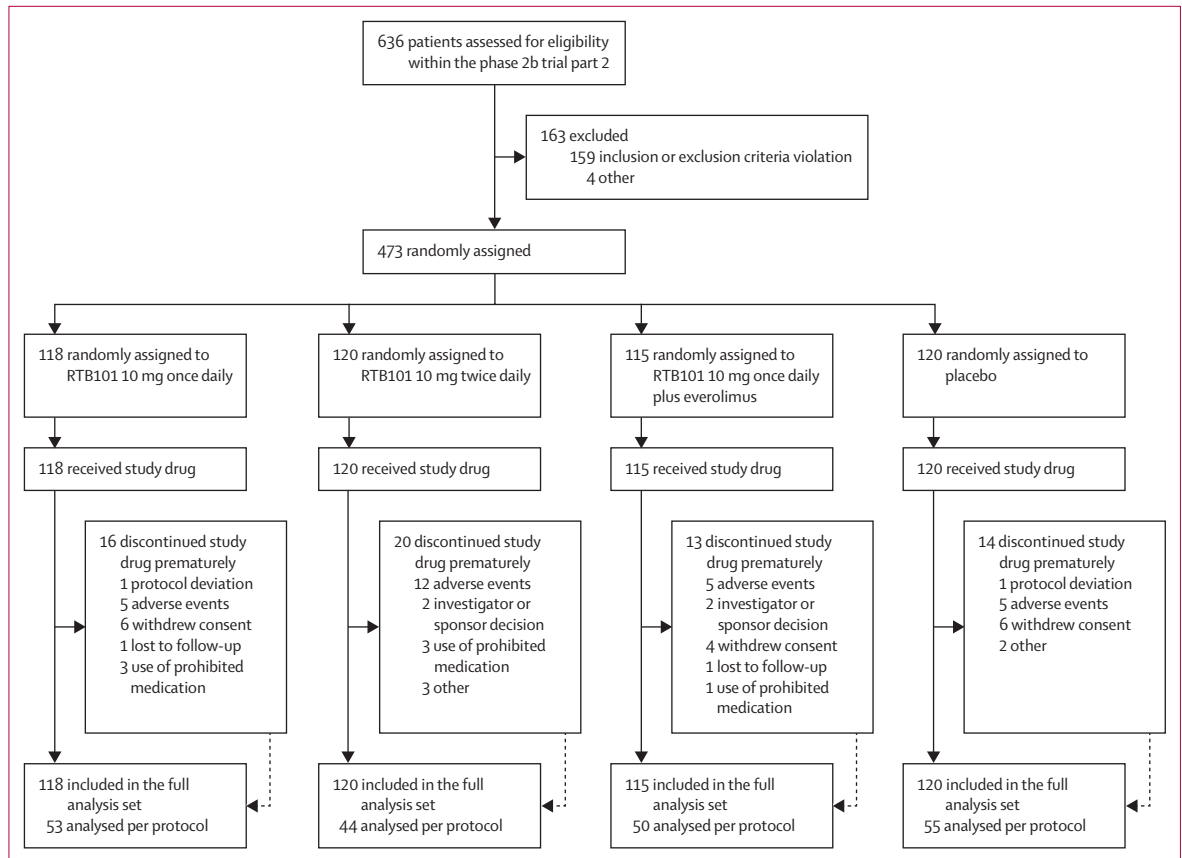
Figure 1: Consort flow diagram for the phase 2b trial

laboratory confirmation of an infection was not relevant to how patients feel and function. Therefore, the FDA proposed a primary endpoint of the proportion of patients with at least one clinically symptomatic respiratory illness, defined as symptoms consistent with an RTI, irrespective of whether the symptoms were confirmed via laboratory testing to be due to an infection.Therefore the primary objective of the study was to investigate whether RTB101 decreased the incidence of clinically symptomatic respiratory illness defined as symptoms consistent with an RTI, irrespective of whether an infection was laboratory-confirmed. The secondary objectives included to investigate whether RTB101 as compared with placebo decreased the proportion of patient with laboratory-confirmed clinically symptomatic respiratory illness; decreased the rate of clinically symptomatic respiratory illness associated with specific viruses; decreased the proportion of patients with severe symptoms due to clinically symptomatic respiratory illness; and the safety and tolerability of RTB101 as assessed by reports of adverse events and serious adverse events, physical examination, electrocardiogram findings, and safety laboratory values. Additional secondary objectives not reported in this manuscript included investigating whether RTB101 as compared with placebo decreased the rate of clinically

symptomatic respiratory illness or the rate of laboratory-confirmed clinically symptomatic respiratory illness, and decreased the time to alleviation of moderate and severe symptoms of respiratory illness.

Statistical analysis
Phase 2b trial
Efficacy analysis for the primary endpoint was done using the full analysis set population, defined as participants who received at least part of one dose of the study drug as treated. For the primary analysis, the proportion of participants with one or more laboratory-confirmed RTIs was compared between each active treatment group and placebo. The primary endpoint was examined via odds ratios (ORs) and 90% CIs for each study drug comparison with placebo. This result was calculated by using a logistic regression including a term for treatment, along with a term for each disease factor and age (continuous) as separate covariates, where a p value for each dose versus placebo was computed on the basis of the estimate of the treatment effect. Two different analyses of the key efficacy endpoints were prespecified. The first analysis accounted for multiplicity by using a fixed-sequence gatekeeping testing procedure that was limited to data from part 2 of the trial and controlled the overall one-sided type I error

rate at 0·05. In this step-down analysis, the comparison of each of the active groups with placebo was ordered as follows: (1) RTB101 10 mg in combination with everolimus 0·1 mg once daily; (2) RTB101 10 mg twice daily; and (3) RTB101 10 mg once daily. All comparisons were done at the same significance level (one-sided α of 0·05), moving to the next comparison only after a significant result was observed for the previous comparison. Because this was an exploratory dose-finding phase 2b trial to discover a dose of RTB101 that led to a greater reduction in laboratory-confirmed RTIs than with placebo, a second analysis of the primary endpoint was prespecified that did not adjust for multiplicity and also used a one-sided α of 0·05. This analysis assessed the proportion of participants with laboratory-confirmed RTIs in each of the active treatment groups compared with in the placebo group and included all data from part 1 and part 2 of the study.

Assuming the following underlying RTI rates (proportion of participants with at least one laboratory-confirmed RTI), which are based on previous data,[10] 39% (placebo) and 23% (active treatment group), a total of 106 participants per group would provide approximately 81% power to yield a statistically significant difference in laboratory-confirmed RTI incidence at week 16, with a one-sided type I error rate of 0·05.

We prespecified an analysis of efficacy in the seven enrolled patient groups. However, two of the patient groups had too few participants to analyse, so we analysed the five remaining groups only.

Phase 3 trial

The proportion of patients with clinically symptomatic respiratory illness (with or without an associated laboratory-confirmed pathogen) beginning at least 3 days after the start of study drug treatment until week 16 was the primary efficacy endpoint for this study. The primary analysis of the primary efficacy endpoint was based on the intention-to-treat principle, comprising all participants who were randomly assigned and had received at least one dose of assigned study drug during the trial (defined as the full analysis set). The primary efficacy endpoint was analysed using a logistic regression model to obtain an estimate of the population OR and associated 95% CIs between RTB101 and placebo. This primary efficacy model was adjusted for factors that might influence response to treatment for patients with clinically symptomatic respiratory illness, including age; frailty score; receipt of current season influenza vaccination; and medical history of asthma, congestive heart failure, or type 2 diabetes. A fixed-sequence gatekeeping strategy was used to control the study-wise error rate at a two-sided α of 0·05. The primary endpoint was tested first at a two-sided α of 0·05.

Sample size was calculated on the basis of a two-sided comparison between RTB101 and placebo from parts 1 and 2 of the phase 2b trial. In the phase 2b trial, 28·2% of participants in the placebo group (excluding participants with COPD and current smokers) had a clinically

	Placebo (n=510)	RTB101 (n=511)	Overall (n=1021)
Age, years			
Mean (SD)	73·1 (5·8)	72·6 (5·8)	72·8 (5·8)
Median (range)	72 (65–91)	72·0 (65–93)	72·0 (65–93)
≥85	24 (5%)	23 (5%)	47 (4·6%)
≥65–<85	486 (95%)	488 (95%)	974 (95·4%)
Sex			
Male	224 (44%)	219 (43%)	443 (43·4%)
Female	286 (56%)	292 (57%)	578 (56·6%)
Ethnicity			
Hispanic or Latino	7 (1%)	1 (<1%)	8 (0·8%)
Non-Hispanic or Latino	503 (99%)	510 (>99%)	1013 (99·2%)
American Indian or Alaska Native	0	0	0
Asian	4 (<1%)	9 (2%)	13 (1·3%)
Black or African American	0	0	0
Native Hawaiian or other Pacific Islander	8 (2%)	11 (2%)	19 (1·9%)
White	498 (98%)	490 (96%)	988 (96·8%)
Other	0	1 (<1%)	1 (0·1%)
Mean height, cm	166·7 (9·9)	167·4 (9·5)	167·0 (9·7)
Mean weight, kg	80·1 (15·7)	81·8 (17·0)	81·0 (16·4)
BMI, kg/m²	28·8 (5·1)	29·2 (5·5)	29·0 (5·3)
Received current season influenza vaccination			
Yes	407 (80%)	415 (81%)	822 (80·5%)
No	103 (20%)	96 (19%)	199 (19·5)

Data are n (%) or mean (SD), unless otherwise specified. Baseline weight and height were defined as the last measurement recorded before the first dose of study medication. BMI=body-mass index. *Age=(date of screening visit–date of birth + 1)/365·25, truncated to complete years.

Table 2: **Phase 3 patient demographics at baseline (full analysis set)**

symptomatic respiratory illness compared to patients in the RTB101 10 mg once daily group, and RTB101 was associated with a 30% reduction in the incidence of respiratory tract infections. This was done as a post-hoc analysis based on the FDA-recommended change in primary endpoint and patient population for the phase 3 trial. With an assumed incidence of clinically symptomatic respiratory illness at week 16 of 28·2% on placebo and of 19·7% in the RTB101 group, a total sample size of 1066 participants (equally randomised) would provide 90% power to detect a 30% reduction in the proportion of participants with clinically symptomatic respiratory illness between RTB101 and placebo using a two-sided test of 0·05 significance. Power analysis was done using a likelihood-ratio χ² test.

Statistical analysis of antiviral gene expression in the phase 2b and 3 trials

The quantitative RT-PCR data (Biomark HD, Fluidigm Corporation) was generated as raw Ct values, with three technical replicates per sample and two samples for each participant (baseline and week 16). The analysis
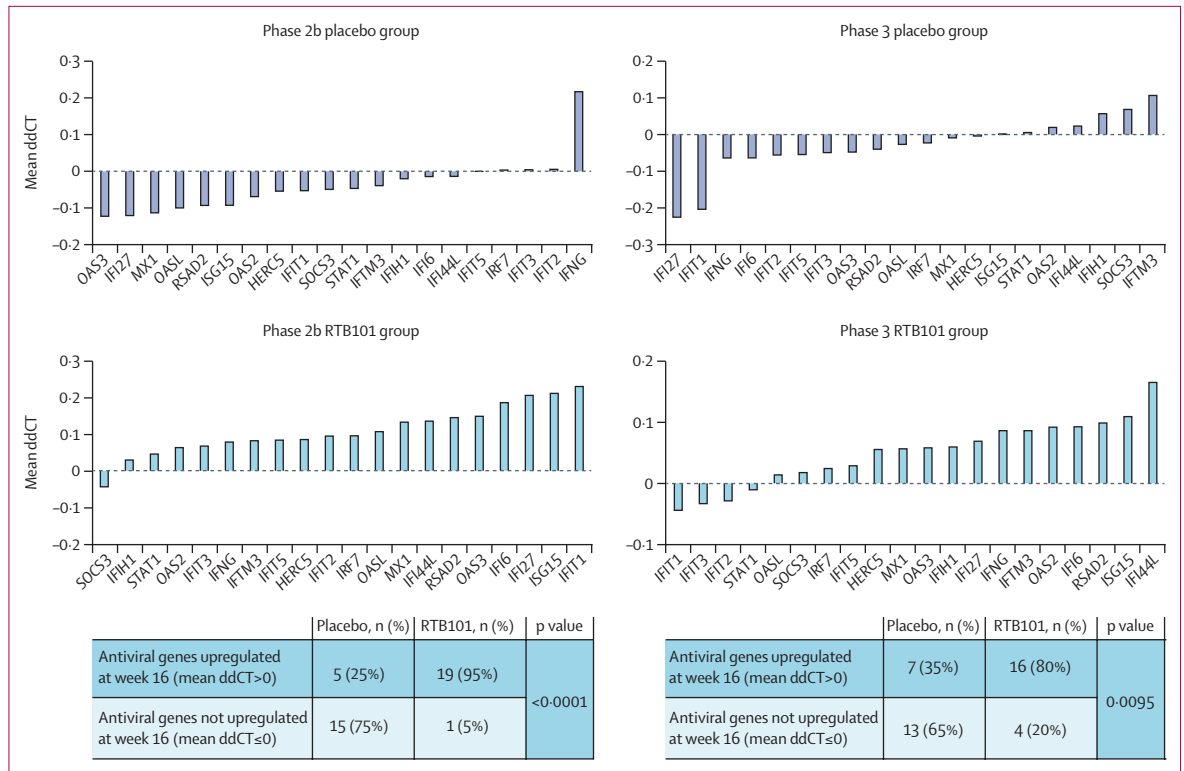
Figure 2: Consort flow diagram for the phase 3 trial
ddCt=delta delta cycle threshold

population included participants with a valid gene expression measurement at both baseline and week 16 for at least one gene of interest and who had given consent to participate in the biomaarker research study. The average Ct for the housekeeping genes was subtracted from the Ct of the gene of interest to calculate the delta Ct (dCt). The delta dCT (ddCt) reflects the change in normalised gene expression over time and was calculated by subtracting the baseline dCT from the dCT at week 16. To assess gene expression change in different groups, a value of "up-regulated" was assigned to a group of participants if the mean ddCT for the gene in that group was greater than zero, and was assigned a value of "not up-regulated" if the mean ddCT for the gene in that group was less than or equal to zero. This value was calculated separately for the placebo and treated groups. Finally, a Fisher's exact test was done to test the difference in the proportion of up-regulated genes in the treated groups compared with the placebo groups.

The phase 2b trial was registered with ANZCTR, ACTRN12617000468325, and ClinicalTrials.gov, NCT03373903. The phase 3 trial trial was registered with ANZCTR, ACTRN12619000628145.

Role of the funding source

This study was administered and sponsored by resTORbio. The funder of the study had a role in study design, data collection, data analysis, data interpretation, and writing of the report.

Results

In the phase 2b trial, 652 participants were recruited between May 16, 2017, and Jan 10, 2018. In part 1 of the phase 2 trial we recruited 179 participants during winter cold and flu seasons in the Southern hemisphere at clinical sites in New Zealand. Participants were randomly assigned 1:1:1 to RTB101 5 mg once daily (61 participants), RTB101 10 mg once daily (58 participants) or matching placebo (60 participants). At the end of Part 1, an interim analysis of the safety and efficacy of each arm in Part 1 was done by an unblinded data monitoring committee. Both RTB101 treatment arms were well tolerated A non-significant reduction in the proportion of patients experiencing one or more laboratory-confirmed RTIs was seen in patients who received RTB101 5 mg once daily (21 [34%] of 61) compared with the placebo group (26 [43%] of 60; OR 0·618 [90% CI 0·325–1·176]; p=0·11). A statistically significant reduction in the proportion of patients experiencing one or more laboratory-confirmed RTIs was seen in those who received RTB101 10 mg once daily (14 [24%] of 58) compared with the placebo group (26 [43%] 60) in part 1 of the study (0·389 [0·195–0·776]; p=0·012). Therefore the data monitoring committee chose RTB101 10 mg once daily as the dose to move forward to part 2 of the study. Only four patients were randomly

assigned to the congestive heart failure stratum and only one patient was randomly assigned to the stratum of one or more emergency room or hospitalisation visits for RTI within 12 months of study entry; thus, prespecified analyses for these subgroups were not done.

In part 2 of the study we recruited 473 patients, who were randomly assigned 1:1:1:1 to RTB101 10 mg once daily (n=118 [25%]), RTB101 10 mg twice daily (n=120 [25%]), RTB101 10 mg in combination with everolimus 0·1 mg daily (n=115 [24%]), or matching placebo (n=120 [25%]).

Baseline demographics between the treatment groups were similar (table 1). Of the 652 patients enrolled, 616 (95%) completed the study (figure 1). Any patient who received one dose of study drug was included in the primary outcome analysis even if they discontinued the study early.

Two statistical analysis of the primary efficacy endpoint were prespecified. The first accounted for multiplicity by using a fixed sequence testing procedure that was limited to data from Part 2 of the trial and controlled the overall type I error. In this step-down analysis, efficacy of RTB101 10 mg in combination with everolimus 0·1 mg once daily compared with placebo was first tested at a one-sided α level of 0·05 and did not meet statistical significance (data not shown). Therefore, the testing procedure was concluded for this analysis. Because this was an exploratory phase 2b dose-finding trial, an additional analysis of the primary endpoint was prespecified that did not adjust for multiplicity. This analysis evaluated the proportion of subjects with laboratory-confirmed RTIs in each of the active treatment groups compared with placebo, and included all data from Parts 1 and 2 of the study. In this analysis we found a statistically significant reduction in the proportion of patients who had one or more laboratory-confirmed RTIs in the RTB101 10 mg once daily treatment group (34 [19%] of 176) compared with the pooled placebo group (50 [28%] of 180; OR 0·601 [90% CI 0·391–0·922]; p=0·025). RTB101 10 mg twice daily and RTB101 10 mg in combination with everolimus 0·1 mg once daily were not associated with a significant reduction in the incidence of laboratory-confirmed RTIs as compared with placebo (data not shown). The results suggest that intermittent inhibition of mTOR (predicted to be achieved with with once daily RTB101 dosing) might be more effective than persistent inhibition (predicted to be achieved with twice daily RTB101 dosing or combination dosing with everolimus) at improving immune function and decreasing the incidence of RTIs.

Secondary endpoint analysis revealed that RTB101 was not associated with a reduced number of patients who had symptoms that met the diagnostic criteria for an RTI, irrespective of whether or not an infection was laboratory-confirmed compared to placebo (56 [32%] of 176 in the RTB10110 mg once daily group vs 68 [38%] of 180 in the placebo group; OR 0·756 [90% CI 0·521–1·098]; p=0·11).
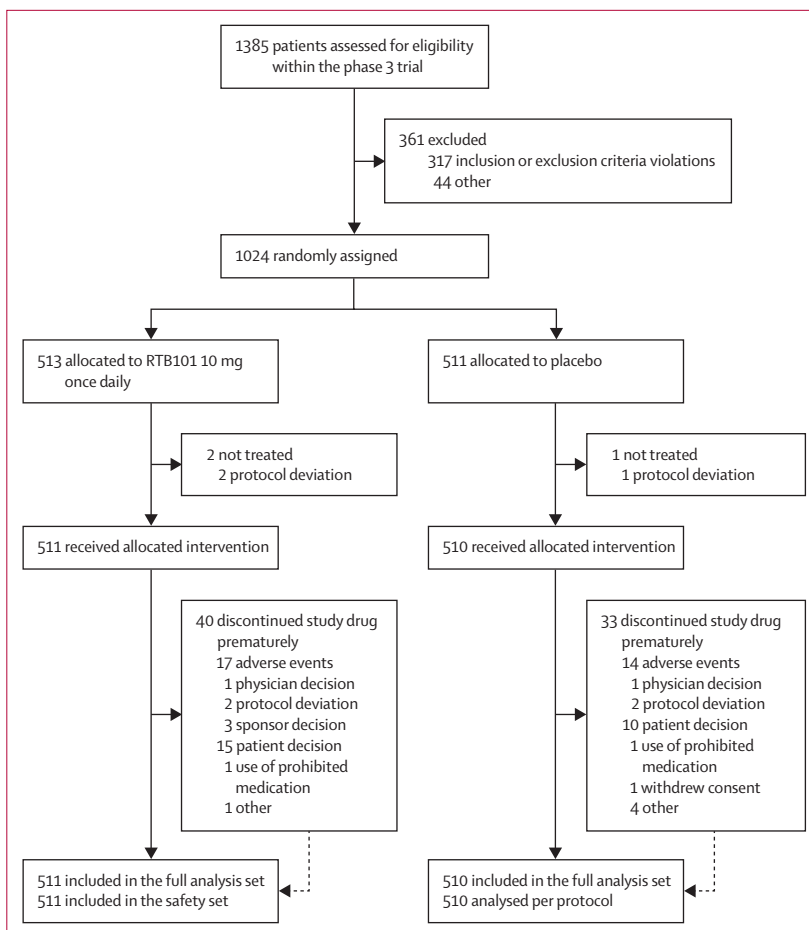

Figure 3: Change in interferon-induced antiviral gene expression from baseline to week 16 in patients treated with RTB101 versus placebo in the phase 2b and phase 3 trials
RNA was isolated from whole blood obtained from patients at baseline and after 16 weeks of study drug treatment and the expression of 20 different ISGs were measured by quantitative PCR. The graphs show the change in expression of each gene from baseline to week 16 in the placebo group (black) and in the RTB101 10 mg once daily group (blue) for both the phase 2b and phase 3 trials. The number and percentage of ISGs upregulated or not upregulated from baseline to week 16 in each treatment group as assessed by ddCt are shown along with associated p values (Fisher's exact test). ddCT=delta delta cycle threshold. ISG=interferon-induced antiviral genes.

The proportion of patients in each group who had a laboratory-confirmed RTI with severe symptoms was also assessed. The RTB101 10 mg once daily group had a reduced proportion of patients who had laboratory-confirmed RTIs with severe symptoms as compared with the placebo group (17 [9%] of 180 in the placebo group and eight [5%] of 176 in the RTB101 treatment group; OR 0·44 [90% CI 0·21–0·92]; p=0·034).

A prespecified analysis of the five patient groups (patients aged at least 85 years or patients aged at least 65 years with asthma, type 2 diabetes, COPD or current smokers) enrolled in the study was done to elucidate whether the efficacy of RTB101 10 mg once daily varied between patient groups. RTB101 10 mg once daily was observed to have consistent treatment effects in part 1 and part 2 of the trial for each patient group (appendix p 2). RTB101 10 mg once daily had the greatest treatment benefit

See **Online** for appendix
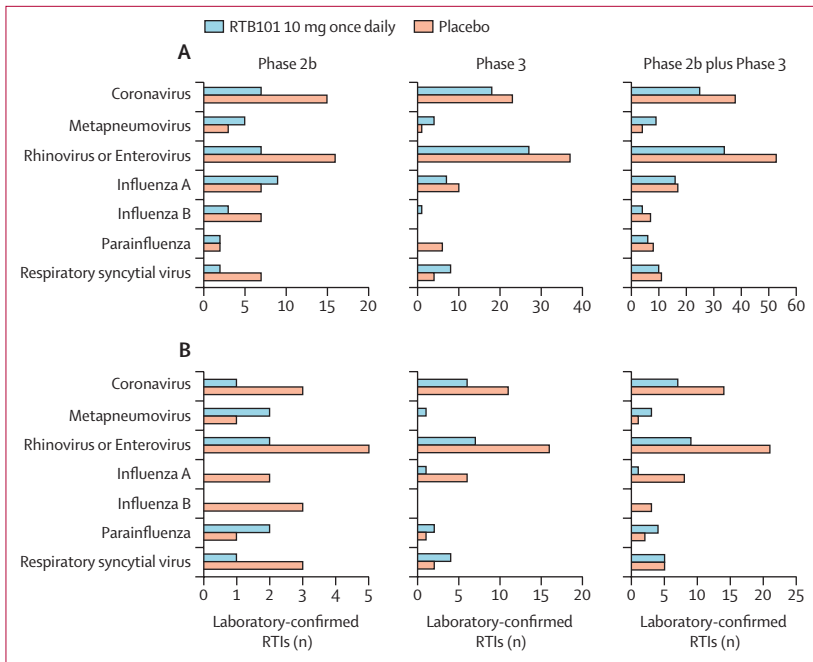
Figure 4: Number of laboratory-confirmed RTIs in each treatment group caused by specific viruses in the phase 2b and phase 3 trials

Part A shows the number of patients with laboratory-confirmed RTIs of any severity caused by specific viruses in the RTB101 group (blue bars) versus the placebo group (grey bars) in the phase 2b, phase 3, and combined phase 2b and phase 3 trials (A). Part B shows the number of patients with laboratory-confirmed RTIs with severe symptoms caused by specific viruses in the RTB101 group (blue bars) versus the placebo group (grey bars) in the phase 2b, phase 3, and combined phase 2b and phase 3 trials. RTI=respiratory tract infection.

in patients who were aged at least 85 years and participants who were aged at least 65 years with asthma (appendix p 2). RTB101 had no benefit in patients with COPD and in current smokers (appendix p 2). Although the incidence of laboratory-confirmed RTIs in current smokers was higher in the RTB101 group than in the placebo treatment group, the incidence of laboratory-confirmed RTIs was low in current smokers and the differences between treatment groups did not meet statistical significance (appendix p 2).

The lack of treatment benefit seen in prespecified analyses in the phase 2b trial in current smokers and in patients with COPD (approximately half of whom were current smokers) is consistent with preclinical data that mTOR inhibitors increase cigarette smoke-induced inflammation.[14,15]

In the phase 3 trial, we enrolled 1024 patients between May 7, 2018, and July 19, 2019. 513 (50·1%) participants were randomly assigned to RTB101 10 mg once daily and 511 (49·9%) to placebo. Baseline demographics between the treatment groups were similar (table 2). Of the 1024 subjects enrolled, 970 (94·7%) completed tudy follow-up irrespective of whether they discontinued study drug early (figure 2).

In the full analysis set, RTB101 did not reduce the proportion of patients with clinically symptomatic respiratory illness, the primary endpoint of the trial (134 [26%] of 511 patients in the RTB101 treatment group vs 125 [25%] 510 patients in the placebo treatment group;

OR 1·07 [95% CI 0·80–1·42]; p=0·65). In this study, a fixed-sequence gatekeeping strategy was used to control the study-wise error rate at a two-sided α of 0·05. Therefore, statistical testing of subsequent clinical endpoints was stopped after the primary endpoint did not meet statistical significance and no further statistical conclusions were made.

The incidence of clinically symptomatic respiratory illness that was laboratory confirmed was assessed as a secondary endpoint. 73 (14%) of 510 patients in the placebo group of the phase 3 trial had a laboratory-confirmed clinically symptomatic respiratory illness, compared to 50 (28%) of 180 patients in the placebo group of the phase 2b trial had a laboratory-confirmed RTI (appendix p 3). Because of the lower than expected incidence of laboratory-confirmed clinically symptomatic respiratory illness in the phase 3 trial, the trial was underpowered for this endpoint. The OR of having a laboratory-confirmed clinically symptomatic respiratory illness in the RTB101 group (65 [13%] 511 patients) as compared with in the placebo group (73 [14%] of 510 patients) was 0·85 (95% CI 0·59–1·22; p=0·38). With a placebo incidence of 14%, we would have needed to randomly assign 2494 patients (1247 patients per group) to have 90% power to detect a 30% reduction at a two-sided α of 0·05.

The incidence of clinically symptomatic respiratory illness with severe symptoms that was laboratory confirmed was also assessed as a secondary endpoint. The proportion of patients with a laboratory-confirmed clinically symptomatic respiratory illness with severe symptoms was reduced in the RTB101 group as compared with in the placebo group (22 [4%] of 511 patients in the RTB101 group vs 31 [6%] of 510 patients in the placebo group; OR 0·70 [95% CI [0·40–1·22]; nominal p=0·21]. The rate of laboratory-confirmed clinically symptomatic respiratory illness with severe symptoms was reduced in the RTB101 group as compared with in the placebo group (23 severe laboratory-confirmed RTIs in 511 patients in the RTB101 10 mg group vs 37 in 510 patients in the placebo group; rate ratio 0·65 [95% CI [0·38–1·11]; nominal p=0·11]).

To determine whether RTB101 increased IFN-induced antiviral gene expression, the expression of 20 IFN-induced antiviral genes was analysed in both the phase 2b and phase 3 trials at baseline and after 16 weeks of study drug treatment in the whole blood of patients treated with RTB101 10 mg once daily or placebo. In both the phase 2b and phase 3 trials, RTB101 was observed to upregulate significantly more IFN-induced antiviral genes as compared with placebo during the 16-week treatment period (figure 3).

To investigate whether upregulation of antiviral gene expression by RTB101 had virus-specific effects, a prespecified analysis of the viruses causing laboratory-confirmed RTIs (figure 4a) and a post-hoc analysis of the viruses causing laboratory-confirmed RTIs with severe

symptoms (figure 4b) in the phase 2b and phase 3 trials were done. Although the numbers of RTIs caused by individual viruses were too low to assess statistical significance, the number of RTIs caused by coronavirus and rhinovirus were consistently lower in the RTB101 group as compared with in the placebo group in both trials (figure 4). Additionally, the number of coronavirus, rhinovirus, and influenza virus infections with symptoms reported by patients to be severe in intensity were consistently lower in the RTB101 group compared with in the placebo group in both trials (figure 4). By contrast, the number of RTIs and severe RTIs caused by metapneumovirus, parainfluenza virus, or respiratory syncytial virus infections were not consistently lower in the RTB101 group as compared with in the placebo group in both trials (figure 4). Of interest, coronaviruses and influenza viruses, but not parainfluenza or respiratory syncytial viruses, have been reported to suppress the host IFN response.[16] Whether upregulation of IFN-induced antiviral gene expression by RTB101 specifically decreases the incidence or severity of RTIs caused by viruses that suppress the host IFN response is unknown.

All dosing regimens were well-tolerated in the phase 2b and phase 3 studies. Overall, the incidence and types of adverse events that occurred in both trials were consistent with what would be expected in the older populations we assesed and we found no clear differences in adverse event profiles between the RTB101 10 mg once daily group and placebo groups (tables 3, 4; appendix pp 4–5). adverse events that occurred in at least 2% of patients in the RTB101 10 mg once daily group as compared with in the placebo group are shown in the appendix (pp 6–7). Patients with serious adverse events were also generally well-balanced between active and placebo groups in both trials (tables 3, 4). Three patients died in the phase 2b trial. One patient in the RTB101 10 mg once daily group died after being hit by a car while riding a bicycle. One patient in the RTB101 10 mg twice daily group and one patient in the placebo group died of unknown causes after the 16-week study drug treatment period. In the phase 3 trial, one patient with type 2 diabetes in the RTB101 10 mg once daily group died of an intracranial haemorrhage. No patients in the phase 2b trial had an serious adverse event that was considered related to study drug treatment, and only one patient in the placebo group in the phase 3 trial had serious adverse events (nausea, fatigue, hyponaetremia, and arthralgia) considered related to study drug treatment.

Discussion

Ageing is caused by a discreet set of biological mechanisms that can be targeted therapeutically as a new way to treat ageing-related conditions.[17] One of the best validated mechanisms underlying ageing biology is the activity of the protein kinase mTOR. Inhibition of mTOR has been shown to extend lifespan and to improve the function of ageing organ systems, including the

	Placebo (n=180)	RTB101 (n=176)
Any AE	149 (83%)	147 (84%)
AE by maximum severity*		
Mild	129 (72%)	131 (74%)
Moderate	73 (41%)	67 (38%)
Severe	14 (8%)	10 (6%)
Study-drug related AE	39 (22%)	35 (20%)
Serious AE	14 (8%)	8 (5%)
AE leading to study drug discontinuation	10 (6%)	9 (5%)
AE leading to study withdrawal	1 (<1%)	2 (1%)
AE leading to death	1 (<1%)	1 (<1%)

Data are n (%). Table shows patients with at least one AE. AE=adverse event. *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.

Table 3: Summary of AEs in the phase 2b trial

	Placebo (n=510)	RTB101 (n=511)
Any AE	371 (73%)	386 (76%)
AE by maximum severity		
Grade 1 (mild)	207 (41%)	213 (42%)
Grade 2 (moderate)	139 (27%)	149 (29%)
Grade 3 (severe)	23 (5%)	21 (4%)
Grade 4 (severe and life-threatening)	2 (<1%)	2 (<1%)
Grade 5 (fatal)	0	1 (<1%)
Study-drug related AE	125 (25%)	117 (23%)
Serious AE	32 (6%)	31 (6%)
Study-drug related serious AE	1 (<1%)	0
AE leading to study drug discontinuation	13 (3%)	18 (4%)
AE leading to study withdrawal	10 (2%)	10 (2%)
AE leading to death	0	1 (<1%)

Data are n (%). Table shows patients with at least one AE.

Table 4: Summary of AEs in the phase 3 trial

immune system, in multiple preclinical species.[18,19] The purpose of our trials was to investigate whether targeting ageing biology with mTOR inhibitors could improve immune function and decrease the incidence of RTIs in older adults at doses that were well tolerated. The mTOR inhibitor RTB101 10 mg once daily for 16 weeks was well tolerated in adults aged at least 65 years, increased expression of IFN-stimulated antiviral genes in peripheral blood, and decreased the incidence of laboratory-confirmed RTIs (the phase 2b primary endpoint), but not the incidence of clinically symptomatic respiratory illness defined as respiratory symptoms consistent with an RTI irrespective of whether an infection was laboratory confirmed (the phase 3 primary endpoint).

Several possible explanations exist for the divergent results of the phase 2b and phase 3 trials, including the change in primary endpoint and changes in the way respiratory symptoms were collected between the two trials. In the phase 2b trial, respiratory illness symptoms were collected during twice weekly telephone calls with patients and the primary endpoint required predefined symptomatic criteria to be met as well as laboratory confirmation of an infection. In the phase 3 trial, respiratory illness symptoms were collected in eDiaries that patients filled out each evening and the primary endpoint was based on symptoms alone without requiring laboratory confirmation of an infection. Multiple investigators in the phase 3 trial anecdotally noted that patients reported in their nightly eDiary respiratory illness symptoms such as cough or headache that were part of the prespecified diagnostic criteria for a clinically symptomatic respiratory illness even when the patient and the investigator did not think that the patient had an RTI. Thus, the occurrence of respiratory symptoms that have non-infectious causes in older adults such as allergies or underlying cardiopulmonary disease might have contributed to the negative result of the phase 3 trial. In support of this hypothesis, RTB101 was also associated with a greater reduction in the incidence of laboratory-confirmed RTIs than the incidence of RTIs diagnosed solely on the basis of respiratory symptoms in the phase 2b trial. Because RTB101 upregulates antiviral gene expression, RTB101 is only likely to reduce the incidence or severity of respiratory symptoms due to viral infections. Laboratory confirmation of an infection might need to be added as a component of the primary endpoint in future trials of therapies like RTB101 that enhance antiviral immune responses in older adults.

Also, upregulation of antiviral gene expression by RTB101 possibly has treatment benefit in only a subset of older adults or a subset of patients with viral infections. The phase 3 trial enrolled a healthier population than that of the phase 2b trial and the healthier adults might have had less attenuation of their type 1 IFN responses and, therefore, obtained less benefit from upregulation of IFN responses by RTB101. Future trials might benefit from the development of biomarkers that identify older adults with deficient IFN responses or at increased risk of RTIs. The results of the phase 2b and phase 3 trials also raise the possibility that RTB101 decreases the incidence or severity of RTIs caused by only a subset of viruses such as coronaviruses that inhibit the host IFN response. To further address this possibility, trials are underway to investigate whether RTB101 prophylaxis decreases the severity of laboratory-confirmed COVID-19 in adults aged at least 65 years.

Of interest, in the phase 2b trial RTB101 was associated with a significant reduction in the incidence of laboratory-confirmed RTIs with severe symptoms. Thus, upregulation of IFN-induced antiviral immunity by RTB101 might have a greater effect on the severity than the incidence of RTIs. Last, the upregulation of antiviral gene expression by RTB101 might be insufficient to decrease the incidence of viral RTIs and the positive results in the phase 2b trial were a result of type 1 error. Arguing against this possibility is the fact that not only in the phase 2b trial but also in two previous phase 2a trials,[10,20] older adults treated with low doses of mTOR inhibitors reported fewer self-reported RTIs than older adults treated with placebo did.

Despite the negative phase 3 results, important lessons were learned from this clinical development programme that is the largest to date targeting ageing biology in humans. First, the results show that it is possible to target mechanisms underlying ageing biology safely with therapies such as mTOR inhibitors in older adults. Second, the results suggest that therapies that target ageing biology in older adults might ameliorate at least some aspects of ageing organ system dysfunction (such as deficient IFN-induced antiviral responses). Further refinement of clinical endpoints and more precise identification of responder patient populations will be important in future trials of therapies that intervene in ageing biology to improve immune function in older adults.

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