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RTB101 and immune function in the elderly: Interpreting an unsuccessful clinical trial

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ABSTRACT

The biopharmaceutical company resTORbio, Inc. Recently announced termination of a phase 3 clinical trial evaluating the ability of the drug RTB101 to improve immune function in the elderly. The company reported that the first stage of the PROTECTOR1 trial did not meet its primary endpoint for reducing clinically symptomatic respiratory illness in healthy older adults. Although RTB101 has been described as an inhibitor of the mechanistic target of rapamycin (mTOR), the PROTECTOR1 trial was not a test of the geroscience hypothesis. Unlike the geroprotective compound rapamycin, RTB101 has not been found to increase lifespan or delay age-related functional or molecular phenotypes in pre-clinical animal models. RTB101 was first developed as an inhibitor of phosphoinositide-3-kinase (PI3K) with secondary inhibitory effects on mTOR. It's ATP-competitive mechanism of action is distinct from the allosteric inhibition by rapamycin and does not specifically target mTOR complex 1 over mTOR complex 2. Clinical development of rapamycin and other specific mTOR complex 1 inhibitors to target age-related indications continues to be robust, and there is growing momentum for translational geroscience, with numerous clinical trials planned or ongoing in this area.

The geroscience hypothesis posits that most, if not all, diseases of aging have shared molecular roots defined by the "hallmarks" or "pillars" of aging [1–3]. One prediction of this hypothesis is that interventions which target these molecular mechanisms of aging will delay or prevent the onset and progression of many age-related functional declines and disorders [4]. As age-related diseases are the largest contributors to disability and death in developed nations, the health and economic implications of the geroscience hypothesis are profound [5].

resTORbio, Inc. is self-described as a "clinical-stage biopharmaceutical company developing innovative medicines that target the biology of aging to prevent or treat aging-related diseases" [6]. Their goal of clinically applying the geroscience hypothesis has been centered around inhibition of the mechanistic Target of Rapamycin (mTOR). mTOR has emerged as a key regulator of aging in laboratory studies, with genetic or pharmacological inhibition of mTOR complex I (mTORC1) having been shown to delay aging in yeast, nematode worms, fruit flies, and several strains of laboratory mice [7,8].

Pre-clinical studies on pharmacological inhibition of mTOR in the context of aging biology have almost exclusively utilized the drug rapamycin or its derivatives, referred to as rapalogs. Rapamycin and rapalogs inhibit mTORC1 by interacting with the FK506 binding protein FKBP12 (FPR1 in yeast), which when bound to rapamycin disrupts mTORC1 [9–11]. Treating mice with rapamycin has been shown to increase lifespan when started early in life [12], late in life [13], when provided intermittently [14], or when given transiently during middle-age [15]. Along with increased lifespan, improvements from rapamycin or rapalog treatment have been reported for age-related cancers [16,17] and age-related declines in cognitive function [18], kidney function [19], heart function [20–22], immune function [23], intestinal function and gut dysbiosis [15,25], ovarian function [26], and oral health [27,28], among others. This large body of literature has suggested multiple potential indications for clinical evaluation of the geroscience hypothesis via treatment with rapamycin or other mTOR inhibitors.

ResTORbio chose to focus their initial efforts on testing whether inhibition of mTOR is sufficient to boost age-related immune function in the elderly. The logic of this approach was based on pre-clinical data showing that middle-aged (24 month) mice treated with rapamycin for 6 weeks are able to mount a significantly

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greater response to influenza vaccine than age-matched control mice [23]. ResTORbio had completed two successful phase 2 trials showing improvement in response to an influenza vaccine and also decreased rates of upper respiratory tract infections in healthy older adults following 6 weeks of treatment with an mTOR inhibitor [29,30]. The positive outcomes from the two phase 2 studies were viewed as highly promising for potential FDA approval.

There are some notable details about the two successful phase 2 trials that are important in order to put the PROTECTOR1 trial into perspective (Table 1). The first phase 2 trial was focused primarily on influenza vaccine response and utilized the rapalog everolimus, which is also known as RAD001. Everolimus has a mechanism of action identical to rapamycin, but with better oral bioavailability and more rapid clearance [31]. The preclinical evidence demonstrating that everolimus can increase lifespan or healthspan in laboratory animals is limited [19,32]; however, given that it works identically to rapamycin, it seems reasonable to expect that everolimus would have effects comparable to rapamycin in pre-clinical models of aging. Thus, the outcome of this trial [29] provided strong evidence for conservation of the pre-clinical studies showing rejuvenation of immune function in mice by rapamycin [23].

The second phase 2 trial expanded upon this result in two important ways. First, the participants were followed for an entire year after the 6 week treatment period, and rates of infection were assessed over that time period. Second, the drug RTB101 (also known as dactolisib or BEZ235) was introduced and tested both alone and in combination with everolimus. RTB101 was initially identified as a phosphoinositide 3-kinase (PI3K) inhibitor that also inhibited mTOR [33] and is among several PI3K/mTOR dual kinase inhibitors that act as ATP-competitive inhibitors in the active site [34]. In addition to its off-target (in this case) effects on PI3K, another important distinction between rapamycin/everolimus and RTB101 is that RTB101 directly inhibits both mTORC1 and mTOR complex 2 (mTORC2). Trends toward reduced rates of infection were seen in all treatment groups compared to placebo and reached statistical significance for the RTB101 alone group and the RTB101 combined with everolimus group [30]. RTB101 alone had no effect on influenza vaccine response in this study, while the combination of everolimus plus RTB101 achieved the primary endpoint of a 1.2-fold increase in antibody titer for all three influenza vaccine strains examined [30]. Everolimus alone significantly improved vaccine response for at least one of the three influenza strains at both doses tested [30], independently replicating the outcome from the first trial [29].

For their phase 3 PROTECTOR1 trial, resTORbio chose to move forward with RTB101 alone and changed the treatment period from 6 weeks to 16 weeks. A total of 1024 healthy adults over the age of 65 were randomized into the study, with half receiving RTB101 and half receiving the placebo [6]. An analysis of the primary endpoint showed that there was no difference between the treatment and placebo groups. It may be that the use of RTB101 alone rather than a rapalog (everolimus), or the change in duration of treatment from 6 weeks to 16 weeks, contributed to the different outcome of the PROTECTOR1 trial. Additional analysis of the trial data will perhaps provide insights into the reasons behind the negative result.

In the context of translational geroscience, it is important to explicitly note that the PROTECTOR1 trial was not a test of the geroscience hypothesis. Unlike rapamycin, there is no data indicating that RTB101 increases lifespan or delays aging in pre-clinical animal models and, as mentioned above, RTB101 is not a specific mTORC1 inhibitor (Table 1). In contrast, the positive results from the resTORbio phase 2 trials with everolimus [29,30] are more directly translatable from preclinical data. While those results are consistent with the geroscience hypothesis, they are not definitive at this point. It seems likely that rapamycin or everolimus can improve immune function in the elderly, but it is uncertain whether efficacy will ever be sufficiently evaluated for FDA approval, due to the prohibitive cost of bringing an off-patent drug (or soon to be in the case of everolimus) through the regulatory process.

Despite the unsatisfying conclusion of the resTORbio respiratory illness program, important lessons have been learned. Feasibility and safety mTOR inhibitors in otherwise healthy people has been established, and there is reason for optimism that mTORC1 inhibitors will eventually be widely used for age-related indications. No significant side effects were associated with everolimus in either of the phase two trials [29,30], and there is accumulating data from other studies demonstrating that rapamycin can be taken safely by healthy older adults [35,36]. This is consistent with a similar lack of side effects in non-human primate marmosets [37] and in older companion dogs treated with rapamycin at doses that may improve age-related heart function [38,39]. A second indication currently being pursued by resTORbio is Parkinson's Disease, where a combination of rapamycin with RTB101 will be evaluated [40]. Alzheimer's disease and related dementias are another area where a large body of preclinical evidence indicates that rapamycin may be effective [41]. The drug is currently being used off-label as a preventative in people at high risk for Alzheimer's disease due to their status as APOE4 carriers [42], and it has been argued that controlled clinical trials for Alzheimer's disease should be vigorously pursued [41]. Another recent study reported that topical rapamycin treatment can reverse signs of skin aging in humans [43], something that will almost certainly be developed further given the huge potential market for such a treatment. In parallel with these efforts toward clinical application, several groups are developing new mTORC1-specific inhibitors in hopes that they will be even more geroprotective than rapamycin [44].

Thus, while the outcome of the PROTECTOR1 trial was disappointing to many in the geroscience field, it does not contradict the geroscience hypothesis, nor does it diminish the likelihood that rapamycin-like mTORC1 inhibitors will be beneficial against aging in people. It is now clear that rapamycin has minimal side effects when taken at lower doses which may be more suitable for delaying or preventing age-related disease, and there are numerous potential indications for mTORC1 inhibitors supported by pre-clinical data. More broadly, the clinical evaluation of geroprotective interventions continues to be robust, including clinical studies of senolytics [45,46], NAD + boosters [47–49], dietary interventions [50–52], metformin [53], and others for safety and initial evidence of efficacy. This trend will undoubtedly continue to accelerate in the next decade, and it seems all but certain that it is only a matter of time before the first clinically validated geroprotective interventions are approved for human use.

Table 1
Comparison of mTOR inhibitors used in preclinical studies of aging and in the resTORbio clinical trials. *Everolimus + RTB101 resulted in a significant reduction in respiratory infections and influenza vaccine response. There was a non-significant trend toward reduced infection in the everolimus alone group [30].

	Rapamycin/Everolimus	RTB101
Allosteric mTORC1 inhibitor	Yes	No
ATP-competitive mTOR inhibitor	No	Yes
PI3K inhibitor	No	Yes
Increases lifespan in mice	Yes	No reports
Rejuvenates Immune function in mice	Yes	No reports
Improves vaccine response in people	Yes (*29*)	No (*30*)
Reduces respiratory infection in people (*30*)	Not significant* (*30*)	Yes (*30*)
Reduces respiratory infection in people (PROTECTOR1 Trial)	Not tested (*6*)	No (*6*)

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