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Authors
Lee, Jong-Hoon Scott
Vo, Thanh-Trang
Fruman, David A

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Targeting mTOR for the treatment of B cell malignancies

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Abstract:	Mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulator of cell growth, division, and survival. Many hematologic malignancies exhibit elevated or aberrant mTOR activation, supporting the launch of numerous clinical trials aimed at evaluating the potential of single-agent mTOR-targeted therapies. While promising early clinical data using allosteric mTOR inhibitors (rapamycin and its derivatives, rapalogs) have suggested activity in a subset of hematologic malignancies, these agents have shown limited efficacy in most contexts. Whether the efficacy of these partial mTOR inhibitors might be enhanced by more complete target inhibition is being actively addressed with second generation ATP-competitive mTOR kinase inhibitors (TOR-KIs), which have only recently entered clinical trials. However, emerging preclinical data suggest that despite their biochemical advantage over rapalogs, TOR-KIs may retain a primarily cytostatic response. Rather, combinations of mTOR inhibition with other targeted therapies have demonstrated promising efficacy in several preclinical models. This review investigates the current status of rapalogs and TOR-KIs in B cell malignancies, with an emphasis on emerging preclinical evidence of synergistic combinations involving mTOR inhibition.

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David A. Fruman, Ph.D.
Professor, Department of Molecular Biology & Biochemistry
Associate Director, UC Irvine Cancer Research Institute
dfruman@uci.edu

UC Irvine, 3242 McGaugh Hall
Irvine, CA 92697-3900
(949) 824-1947 TEL
(949) 824-8551 FAX

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Editorial Office
British Journal of Clinical Pharmacology

Dear Editor,

Thank you for providing reviewer comments and inviting a revised version of our review article, entitled "Targeting mTOR for the treatment of B cell malignancies". We are grateful for the positive feedback and the helpful suggestions. We have prepared a point-by-point reply that addresses all of the concerns raised by the referees as well as the comments of the editor. We have also prepared the revised figures according to the instructions. We hope that these changes are acceptable to the journal.

As requested by the Editorial Office, the name of the Principal Investigator is David Fruman.

Sincerely,

David Fruman

Point by Point Response to Reviewers and Editor
Lee, Vo and Fruman
British Journal of Clinical Pharmacology
RT-00761-15

Referee: 1

Minor suggestions:
1. Page 8: The authors describe studies of mTOR inhibitors (MTIs) with an emphasis upon studies performed in pediatric ALL. Consider citation addition & brief update for everolimus + chemo trials (AE Place ASH 2015 abstract #3765) for most current information.
This has now been updated on page 6, lines 196-199.

2. Reference #167 appears incomplete in citation and should be updated.
This is now reference #172 and has been updated.

3. Figure number is generous. Consider condensing into smaller number of figures, as they are very similar and individually highlight relatively minor points/data. In particular, consider deletion of Figure 5, which adds minimal information above that described in the main text.
To reduce the overall figure number, we chose to remove former Figure 4. We felt that this was largely redundant with previous figures and the new points about PIM and MNK kinases were described sufficiently in the main text. Former Figure 5, now Figure 4, contains conceptual information that we feel is important for the reader to view in Figure format.

4. Table 1: please define ORR in footnote and also on page 18/line 369 if not previously done. Consider changing Table 1 column title "Notes" to "Outcomes" or "Results." Consider adding column for class of inhibitor (e.g., MTI, TOR-Ki, etc.) after the drug names and updating title name.
ORR has been defined in a footnote. It is also defined in the main text on line 213.
Table 1 column 6 title has been changed to "outcomes".
A column has been added for drug class.
We did not alter the main title of the Table, which seems to adequately describe the content: "Published trials of mTOR-targeted therapies in ALL and NHL"

Typographical corrections:
1. page 6, line 19: change "relapse" to "relapsed"
Fixed. This is now on page 5, line 167.

2. Please remove erroneous commas placed before non-independent clauses in compound sentences.
We have checked the text thoroughly and attempted to remove unnecessary commas.

Referee: 2

Minor points:
1. Highlighting or circling the mTORC1 and mTORC2 complexes in Figure 1 will make the figure more understandable.
Boxes have been placed around mTORC1 and mTORC2 in a revised version of Figure 1.

Provide a brief background explanation of how mTOR senses ATP and amino acids to maintain cellular homeostasis. Explain in detail the regulation sequence between mTORC1, 4EBP1 and eIF4E. Add an explanation of PDCD4 function in mTOR signaling.
These requests are all addressed in the revised text, page 3, lines 59-72. We also briefly expanded the description of mTORC2 regulation on page 2, lines 45-48.

2. Include FKBP12 along with rapalogs in Figure 2 and add an inhibiton arrow directed at mTORC1.
This has been added.

3. The title “Rapalogs: partial mTORC1 inhibitors” (line 91) should be replaced with “ Rapamycin and Rapalogs: partial mTORC1 inhibitors”; the subtitle “Rapalogs in B-ALL” (line 103) should be replaced with “ Rapamycin and Rapalogs in B-ALL”.
Done.

4. Add reference after “…in vitro or in xenograft models” (line 110).
Three new references have been added here. Now line 157.

Executive Editor's comments:
Executive Editor
Comments to the Author:
Please revise your manuscript according to the comments of the reviewers. Thank you.
Done, please see above.

Comments Regarding Format from the Editorial Office:

1) The submission guidelines for the British Journal of Clinical Pharmacology have changed slightly. We now request a brief statement in the cover letter which clearly states the name of the Principal Investigator.
This statement have been added to the cover letter.

2) Abstract: A structured summary must appear before the Introduction and include the following headings: Aim(s), Methods, Results (some numerical data, including confidence intervals on differences, when appropriate, must be included), Conclusions
The summary should be a maximum of 250 words. Please ensure the summary within the manuscript matches the one requested in the separate box during submission.

This submission is a review article, not a research study. Therefore there were no aims, methods or results to include in a structured summary. We have included an abstract of 176 words. We believe this might have been overlooked because it was labeled "summary" rather than "abstract", and because there was no header for the next section. We have now added "Introduction" as a header to the section immediately following.

3) Please amend your conflict of Interest Statement. The statement should follow the format used by the British Medical Journal (BMJ) and must contain all three of the statements included below:

"All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work OR [author initials] had support from [name of organisation] for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years OR [author initials] [had specified relationship] with [name of organisation] in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work OR [initials of relevant authors] [had specified relationships or activities of this type]"
An appropriate Conflict of Interest statement has been added after the acknowledgements, before the References. All authors have completed the Unified Competing Interest form.

4) Title Page:
– title should give an informative and accurate indication of the content of the paper. It should be no longer than 150 characters (including spaces);
This was already present; no changes have been made. The title is 55 characters, including spaces.

– a running head of no more than 75 characters, including spaces
The title page now provides a running title of 38 characters:
"mTOR inhibitors in B cell malignancies"
Please note that the main title is less than 75 characters so this could be used as well.

– keywords (these are used to identify potential referees and as indexing terms)
These were already present; no changes have been made.

– the word count, excluding the title page, summary, references, tables, and figures
– the numbers of tables and figures.
These have been added.

5) Figure Files: Please upload files as GIF, JPEG, TIFF or PICT files [images >300dpi and graphs >600dpi]. PDFs and PPTs are not accepted. This is because should your manuscript be accepted for publication the Production Editor will need to edit the files in order to prepare them for print. *Figures in the request resolution have been provided as .jpg files*

Tables: Please upload tables as DOC or EXCEL files which are editable. Do not embed the tables as pictures. *The Tables are uploaded as .docx files in landscape view.*

Targeting mTOR for the treatment of B cell malignancies

J. Scott Lee, Thanh-Trang Vo, and David A. Fruman

Department of Molecular Biology & Biochemistry, University of California, Irvine

Corresponding Author:

David A. Fruman, PhD

3242 McGaugh Hall

University of California, Irvine

Irvine, CA 92697

Tel: (949) 824-1947

Fax: (949) 824-8551

Email: dfruman@uci.edu

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Word count: 4,574

Figure count: 4

Table count: 2

Abstract

Mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulator of cell growth, division, and survival. Many hematologic malignancies exhibit elevated or aberrant mTOR activation, supporting the launch of numerous clinical trials aimed at evaluating the potential of single-agent mTOR-targeted therapies. While promising early clinical data using allosteric mTOR inhibitors (rapamycin and its derivatives, rapalogs) have suggested activity in a subset of hematologic malignancies, these agents have shown limited efficacy in most contexts. Whether the efficacy of these partial mTOR inhibitors might be enhanced by more complete target inhibition is being actively addressed with second generation ATP-competitive mTOR kinase inhibitors (TOR-KIs), which have only recently entered clinical trials. However, emerging preclinical data suggest that despite their biochemical advantage over rapalogs, TOR-KIs may retain a primarily cytostatic response. Rather, combinations of mTOR inhibition with other targeted therapies have demonstrated promising efficacy in several preclinical models. This review investigates the current status of rapalogs and TOR-KIs in B cell malignancies, with an emphasis on emerging preclinical evidence of synergistic combinations involving mTOR inhibition.

Introduction

The mTOR Signaling Pathway

mTOR is a serine/threonine kinase that functions as a master regulator of cell growth, proliferation, metabolism, and survival. mTOR is active in two distinct multi-protein complexes (mTORC1 and mTORC2) that are characterized by the defining subunits RAPTOR and RICTOR respectively [1,2]. Each complex is differentially regulated and has a distinct set of substrates (Figure 1). Activation of mTORC2 is incompletely understood, but has recently been shown to be dependent on the generation of $PI(3,4,5)P_3$ by phosphoinositide 3-kinase (PI3K) [3]. Upon activation mTORC2 functions to amplify the activity of AKT, a key oncogene involved

in cell survival and metabolism [4,5]. On the other hand, mTORC1 activation is coordinately regulated by growth factor signals (i.e. from the PI3K/AKT pathway), nutrient availability (amino acids), and cellular energy status (ATP levels). Under conditions of low nutrients, amino acid sensors (such as SLC38A9 [6,7]) suppress mTORC1 activation. Similarly, under conditions of low energy (low ATP), 5' AMP-activated protein kinase (AMPK) can also suppress mTORC1 activation [8]. This multifaceted regulation ensures that the cell is at an appropriate bioenergetic state to support cell growth and division [9,10] (Figure 1).

Upon activation, mTORC1 promotes key biosynthetic pathways including translation, transcription, and lipogenesis, while suppressing apoptotic and autophagic processes [11,12]. The most well-characterized downstream targets of mTORC1 are the p70 ribosomal-S6 kinases (S6Ks) and eukaryotic initiation factor 4E (eIF4E) binding proteins (4E-BPs). Phosphorylation of S6Ks induces its activity, which is critical for lipid and ribosome biogenesis pathways and promotes translation via suppression of PDCD4 and activation of eIF4B [13,14]. In contrast, phosphorylation of 4E-BPs suppresses their ability to inhibit eIF4E, which promotes translation initiation [15]. Together, these effectors coordinately increase protein synthesis rates, a process whose dysregulation is a central driving mechanism in cancer [16,17]. Importantly, hyper-activating mutations in mTOR itself have been identified in many cancers and further indicates the importance of mTOR activity to tumorigenesis [18].

Evidence of mTOR activation in B-ALL and NHL

Aberrant activation of mTOR is frequently associated with poorer prognosis and has been well described in B cell malignancies including B cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkin's lymphoma (NHL). Given that mTOR is a convergence point for many distinct signaling pathways, there are many mechanisms by which it may become inappropriately activated (Figure 2). In B-ALL, the most common mode is through activation of upstream kinases. For example, the Philadelphia chromosome (Ph+), characterized by the BCR-ABL

translocation, induces robust activation of several parallel pathways leading to mTOR activation. Similarly, genomic profiling has recently identified a Ph-like subset of B-ALL, which exhibits a similar kinase activation signature to that of Ph+ B-ALL. Notably, these mutations are strongly associated with poorer outcomes in both children and adults [19-22]. Empirical evidence has also shown a direct correlation between AKT and/or mTOR activation and poor prognosis in patients with pediatric and adult B-ALL [23-25].

Among NHL subtypes, activation of mTOR is consistently a reliable indicator of more aggressive disease and poorer prognosis [26-30]. Similar to B-ALL, activation of mTOR follows through direct mutations in key upstream pathways. In mantle cell lymphoma (MCL), amplification of *PIK3CA* (the gene encoding the catalytic subunit of PI3K) and/or PTEN loss (the negative regulator of PI3K activity) have been observed in a large fraction of primary tissue samples [31]. In diffuse large B cell lymphoma (DLBCL), activation may be similarly achieved via mutations in *PIK3CA* [32,33] or chronic B cell receptor activation [34]. In follicular lymphoma (FL), mTOR is aberrantly activated by way of PKCζ or Syk kinases [35-38]. Collectively, these data highlight the impact of elevated mTOR activity on patient outcomes, and provide a solid rationale for the use of mTOR-targeted therapies in these B cell malignancies.

Rapamycin and Rapalogs: partial mTORC1 inhibitors

Mechanism of action

Upon entry into a cell, rapamycin binds to FKBP12 forming a complex that potently and selectively suppresses mTORC1 kinase activity by limiting substrate access to the active site [39,40]. Importantly, the rapamycin-FKBP12 complex cannot bind to mTORC2 [2,41], though in some cases prolonged exposure may limit the assembly of mTORC2 [42]. In this manner, rapamycin behaves as a highly potent and selective inhibitor of mTORC1 (Figure 3). However, poor solubility and pharmacokinetics spurred the development of rapamycin analogs (termed rapalogs) for oral dosing in cancer patients [43]. Most notable among these rapalogs are

temsirolimus (CCI-779, Wyeth Pharmaceuticals [44]), everolimus (RAD001, Novartis Pharmaceuticals [45]), and ridaforolimus (AP23573, Merck and ARIAD Pharmaceuticals [46]).

Rapamycin and Rapalogs in B-ALL

Early testing with rapamycin unveiled potent anti-proliferative efficacy in several preclinical models of ALL. In an Eμ-RET model of murine B-pre ALL, rapamycin as a single agent potently inhibited proliferation of leukemia cells both *in vitro* and *in vivo* [47,48]. Similar efficacy was later observed in models of Ph+ B-ALL [49,50] as well as in Ph-like B-ALL driven by JAK pathway mutations or CRLF2 rearrangement [51]. Rapalogs also demonstrated marked preclinical efficacy in primary human ALL samples grown *in vitro* or in xenograft models [50-52]. Notably, rapamycin demonstrated single-agent cytotoxicity in primary pediatric ALL samples and sensitized cells to doxorubicin *in vitro* [52]. Both everolimus and temsirolimus have shown similar efficacy in xenograft models of adult and pediatric primary human ALL as single agents [53] and in combination with chemotherapy [54,55].

Clinically, rapamycin as a single agent exhibited no dose-limiting toxicities, but had lackluster efficacy compared to standard chemotherapeutic options (Table 1). In an early trial, rapamycin yielded stable disease in only three out of nine pediatric patients with relapsed ALL [56]. As a result, several trials have been launched to determine whether rapalogs can combine safely and effectively with standard chemotherapies. An early pilot trial combining rapamycin with glucocorticoids in relapsed ALL patients found that rapamycin effectively reduced the anti-apopotic protein MCL-1 in various patients. This promising outcome suggested that rapamycin might sensitize ALL cells to apoptosis-inducing drugs. Indeed, in another study combining temsirolimus with intensive multi-drug re-induction therapy (dexamethasone, mitoxantrone, vincristine, and PEG-asparaginase) in relapsed childhood ALL yielded complete response in seven of sixteen patients, of which three had less than 0.01% minimal residual disease (MRD) by the end of treatment [57]. However, a separate trial evaluating everolimus combined with

intensive chemotherapy (hyper-CVAD) in relapsed B-ALL yielded complete remission rates that were similar to standard salvage chemotherapies (~35%) [58-60]. These trials highlight how the efficacy of rapalogs seem to be dependent on which chemotherapeutics are used, warranting further investigation.

A key question that remains to be answered is whether rapalogs combined with chemotherapy will demonstrate acceptable toxicity profiles. In the aforementioned trial combining temsirolimus with re-induction chemotherapy, the treatment was associated with unacceptable toxicities including severe infections that led to one death due to sepsis [57]. However, a recent multi-center study testing the combination of everolimus with prednisone, vincristine, PEG-asparaginase and doxorubicin demonstrated that the combination was well tolerated in pediatric patients with first relapse [61]. Further trials are being performed, including an expansion of the aforementioned trial as well as one testing the safety of temsirolimus with less intensive re-induction (etoposide and cyclophosphamide; NCT01614197). Together, these results show that rapalogs have some potential in combination therapy, but an effective and tolerable regimen in B-ALL has yet to be identified. Moving forward, it will be important to identify which chemotherapeutics are best combined with rapalogs and whether modifications to the dose and/or schedule may alleviate dose-limiting toxicities.

Rapalogs in NHL

Similar to B-ALL, preclinical testing of rapalogs in NHL revealed promising cytostatic effects both *in vitro* and *in vivo*, yet clinical responses were limited in most contexts. For example, in MCL, FL, and DLBCL, rapamycin potently suppressed the proliferation of cell lines and primary patient cells *in vitro* [62-66]. However, the clinical use of rapalogs has only made progress in MCL where responses to standard chemotherapies are limited (Table 1). In phase II trials of relapsed MCL, single agent administration of either temsirolimus or ridaforolimus yielded overall response rates (ORR) of 38% [67] and 33% [68] respectively. Notably, a subsequent phase II

trial using a 10-fold lower dose of temsirolimus revealed that similar responses could be obtained with lower toxicity [69]. Based on these results, a randomized phase III trial was conducted. Strikingly, the ORR and progression free survival were significantly higher in patients treated with temsirolimus compared to investigator's choice agent. These results ultimately led to approval for temsirolimus as a single agent therapy for relapsed/refractory MCL in Europe [70]. A subsequent phase II trial has also been completed combining temsirolimus with rituximab in relapsed/refractory MCL. Despite demonstrating higher response rates than single agent temsirolimus, the combination was also associated with higher toxicities including thrombocytopenia and neutropenia in a significant fraction of patients [71]. Rapalog monotherapy has also elicited responses in a subset of patients with other NHL subtypes. In a phase II trial of everolimus in relapsed lymphoma, the ORR in DLBCL was 30% (14/47) and 38% (3/8) in FL [72]. Similar results were seen with temsirolimus where the ORR was 28% for DLBCL and 53% in FL [73]. While these studies highlight that rapalogs have some activity, the availability of better therapeutic options in both DLBCL and FL has limited the clinical progress of rapalogs in these diseases. Thus, across NHL subtypes it will be important to determine whether the addition of rapaogs to standard chemotherapy can provide additional benefit to patients, without increasing toxicities.

Outlook:

Overall, despite showing promising preclinical activity in hematologic malignancies, rapalogs have only gained regulatory approval for use in one disease setting (MCL) where standard chemotherapies have limited efficacy. A major issue is that rapalogs given as single agents tend to elicit primarily cytostatic responses in hematologic malignancies [62,63,66,74]. Clinically, the lack of inherent cytotoxicity is problematic since discontinuation of treatment may permit tumor cell regrowth [75-77]. While continued treatment may combat this issue, whether rapalogs at anti-leukemic doses will be safe for long-term use also remains to be seen. Clinical evidence of

several toxicities including thrombocytopenia, mucositis, and hyperlipidemia suggests that prolonged treatment will be difficult to manage [43]. Alternatively, combinations with chemotherapy are actively being investigated and may reposition rapalogs as an adjuvant to improve chemotherapeutic responses. On this note, it is important to point out that the cytostatic activity of rapalogs will likely limit its potential to combine with certain chemotherapies, necessitating the identification of cytotoxic drugs that will synergize with rapalogs productively while maintaining acceptable tolerability.

While rapalogs provided proof-of-concept for effective mTOR targeted anti-cancer therapies, they exhibit many unfavorable biochemical properties that may also limit their clinical potential. Most notably, failure to suppress mTORC2 kinase activity allows maintained survival signaling through AKT and other related kinases. This issue is exacerbated by the existence of a negative feedback loop downstream of mTORC1 (Figure 3). Selective inhibition of mTORC1 induces robust feedback activation of upstream PI3K/AKT and MAPK pathways allowing cancer cells to escape from the effects of rapamycin [57,78-82]. Additionally, rapalogs are known to incompletely inhibit the phosphorylation of a subset of mTORC1 substrates (Figure 3). Despite restricting access to the active-site, rapalog-induced suppression of 4E-BP1 phosphorylation is refractory to long-term treatment compared to phosphorylation of p70S6K [83]. The cause of this differential sensitivity has recently been attributed to distinct substrate sequences near the phosphorylation sites [84]. This incomplete suppression of mTORC1 may significantly impact the anti-cancer potential of rapalogs as sustained activation of eIF4E is known to promote oncogenesis [85]. Consequently, sustained 4E-BP phosphorylation may allow cancer cells to escape from rapamycin-induced cell cycle arrest [86]. Thus, more complete mTOR inhibition may be required to elicit more promising clinical responses.

TOR-KIs: complete mTORC1/2 inhibitors

The timely development of mTOR kinase inhibitors (TOR-KIs) directly addressed the biochemical disadvantages of rapalogs. By competing with ATP for binding to the mTOR active site, not only do TOR-KIs more completely block mTORC1 substrate phosphorylation (namely 4E-BPs), but they also inhibit mTORC2 activity [87,88]. This results in reduced phosphorylation of AKT at Ser473 (Figure 3), dampening the feedback activation of PI3K/AKT that is known to limit rapalog efficacy [89-91]. It is important to note that by competing with ATP, TOR-KIs are capable of inhibiting several kinases at higher doses, including the structurally related protein, PI3K. Conversely, several compounds that are often used pre-clinically as PI3K inhibitors (wortmannin, LY294002) directly inhibit mTORC1 and mTORC2 at similar concentrations. Thus, it is important to fully understand the pharmacologic properties of ATP-competitive mTOR and PI3K inhibitors when interpreting their preclinical and clinical efficacy.

Several structurally distinct mTOR-selective inhibitors have been reported and tested in models of B cell malignancies. Most notable among them are PP242 [88], Torin1 [87], Ku-0063794 [92], AZD8055 [93], AZD2014 [93], MLN0128 (previously INK128 [94]), and CC-223 [95]. In preclinical testing, these TOR-KIs proved superior to rapalogs in terms of cytostatic and cytotoxic potential. For example, in a mouse model of AKT-driven lymphangiogenesis, PP242 strongly suppressed both 4E-BP1 phosphorylation and tumor growth compared to rapamycin [96]. These findings were also recapitulated *in vitro* using leukemia and DLBCL cell lines where TOR-KIs had a greatly improved biochemical effect on downstream 4E-BP phosphorylation [97-99].

Despite the broader biochemical impact of TOR-KIs over rapalogs, whether complete mTOR kinase inhibition is sufficient to elicit cytotoxic responses is yet to be established. Two reports of structurally distinct TOR-KIs in B-ALL demonstrated that mTOR kinase inhibition was sufficient to induce apoptosis in B-ALL cell lines compared to rapamycin [100,101]. However, in both studies, apoptosis was only observed at doses of TOR-KI that greatly exceed what was needed to fully suppress mTOR kinase activity as measured by western blot. At lower doses that still

fully suppress mTOR activity, our lab has found that both AZD8055 and MLN0128 maintain a primarily cytostatic response profile (that is greater than rapalogs) [98,102-104]. Notably, low doses of PP242 were sufficient to kill murine bone marrow cells immortalized by p190-BCR-ABL [99], suggesting that fully transformed B-ALL cells with additional oncogenic lesions may respond differently to mTOR inhibition. Thus, it remains unclear whether TOR-KIs will be effective in B-ALL or NHL as a single agents at doses that are highly selective for mTOR kinase activity.

Early clinical trials have suggested that while TOR-KIs are more effective than rapalogs at suppressing tumor growth, they may also be less tolerable [78]. A single agent tolerability test of AZD2014 showed dose-limiting toxicities that were similar to rapalogs including mucositis and fatigue [105]. Both CC-223 and MLN0128 also presented similar toxicities, but hyperglycemia also occurred and necessitated close monitoring of patient blood [106,107]. Several additional clinical trials are currently in progress to address the efficacy and tolerability of TOR-KIs and are summarized in **Table 2**. However, a key question is to investigate whether TOR-KIs will retain anti-cancer efficacy at lower doses that minimize these toxicities. While it is likely that lowering the dose of TOR-KIs may improve their tolerability, it will also impinge on their ability to fully suppress mTOR kinase activity. Moving forward, it may be important to determine whether these potentially suboptimal doses, which only partially inhibit mTOR, will be more effective than clinically tolerable doses of rapalogs, which potently inhibits phosphorylation of some, but not all, mTORC1 substrates.

Emerging Combinations with mTOR Inhibitors:

Recent research efforts have been dedicated to identifying promising combinations that can synergistically kill cancer cells. The rationales behind these emerging combinations can be loosely categorized into two broad groups. The first approach seeks to exploit known resistance mechanisms to mTOR inhibition; either by targeting feedback pathways or using apoptosis-

sensitizing agents. The second approach seeks to evaluate the potential of mTOR inhibitors as adjuvants to augment the effects of other agents targeting known oncogenic drivers. While both approaches have yielded several promising combinations, whether they can be translated to significant clinical responses with acceptable toxicity still remains to be determined.

Combinations targeting resistance mechanisms

Targeting parallel and downstream pathways

As with all targeted therapies, an understanding of how cells maintain survival in the presence of mTOR inhibitors has been crucial to the identification of promising combinations. Currently, there are several known acquired and *de novo* mechanisms of resistance to mTOR-targeted therapies. For example, in addition to feedback activation of PI3K/AKT, mTORC1 inhibition may also activate the parallel MAPK/ERK pathway in B-ALL. In a similar fashion, PI3K/AKT/mTOR inhibition can also induce up-regulation of receptor tyrosine kinases (RTKs) leading to resistance in some solid tumors [108]. In agreement with these induced resistance mechanisms, the addition of MAPK inhibitors and RTK inhibitors have demonstrated significantly more efficacy in combination with both rapalogs and TOR-KIs in preclinical settings [80,109,110]. However, in other instances resistance to mTOR inhibition may be a result of sustained downstream effector activity, particularly cap-dependent translation. For example, our laboratory has noted resistance to TOR-KIs in DLBCL cell lines lacking expression of 4E-BPs [98] or over-expressing eIF4E [111]. Furthermore, recent evidence has indicated that PIM and MNK kinases can maintain cap-dependent translation downstream of mTORC1 inhibition [112]. In these situations, targeting cap-dependent translation indirectly using combinations of PIM or MNK inhibitors with TOR-KIs has shown cytotoxic activity in AML cell lines [113,114] as well as in cutaneous T cell lymphoma cell lines *in vitro* [115]. Additional work is required to evaluate the potential of directly targeting the cap-dependent translation initiation machinery. It is likely that

other mechanisms of resistance will arise as our experience with mTOR inhibitors increases, and these may ultimately support the study of additional combinations.

While clinical data regarding the efficacy of these combinations in B cell malignancies has not reached maturity, similar combinations have been successfully deployed in non-hematologic malignancies. For example, inhibition of the upstream tyrosine kinase, HER2, significantly improved the efficacy of mTORC1/2 inhibition in patients with refractory breast cancer compared to single agent treatment [116]. Similarly, combinations of PI3K/AKT/mTOR and Ras/MAPK/ERK pathway inhibition yielded improved response rates in patients with advanced refractory solid tumors, but did so at the cost of significantly higher toxicities [117]. Collectively these studies highlight the potential of using mTOR inhibitors in combination with agents targeting known resistance pathways to mTOR inhibition or as an adjuvant therapy to augment the effects of other rational targeted therapies. However, it will be important to determine whether these combinations targeting multiple key survival pathways will remain selective for cancer cells as toxicity will be a major concern.

Targeting apoptosis

Another straightforward approach to directly enhance the apoptotic potential of mTOR inhibition is to target the pro-survival BCL-2 family proteins. Apoptosis is regulated through dynamic and competitive binding interactions between anti-apoptotic proteins (e.g. BCL-2, BCL-XL, BCL-w, and MCL-1) and pro-apoptotic sensitizers (e.g. BAD, PUMA, and NOXA), activators (e.g. BIM and BID), and effectors (BAX and BAK) (Figure 4). While mTOR inhibition is known to suppress survival signaling through both mTORC1 (e.g. MCL-1 expression [96]) and AKT (e.g. inhibition of BAD and down-regulation of BIM [118,119]), TOR-KIs are insufficient to induce apoptosis through this pathway. Thus, a simple approach would be to use antagonists of the pro-survival proteins to disrupt their binding capacity, and subsequently lower the threshold for BIM to activate BAX/BAK-mediated MOMP and apoptosis [120].

ABT-737, and its orally bioavailable analog, ABT-263, represent the most potent and selective small molecule inhibitors of BCL-2 and BCL-X_L. Both of these compounds demonstrated remarkable cytotoxic potential that was significantly enhanced when combined with mTOR inhibitors in DLBCL [121], FL [122], AML [123], and B-ALL [124]. However, due to on-target toxicity associated with BCL-X_L inhibition [125], a more promising clinical candidate is ABT-199 [126]. ABT-199 is a selective inhibitor of BCL-2 and has elicited substantial clinical responses in patients with CLL as a single agent [127], leading to its designation as a breakthrough therapy for CLL patients with a 17p deletion (p53). Importantly, we and others have recently reported that ABT-199 synergizes with mTOR inhibition comparably to dual BCL-2/BCL-X_L inhibitors [104,128], suggesting that the rationale established using first generation BCL-2 antagonists will hold true for ABT-199. However, a key concern is whether the addition of TOR-KIs to BCL-2 antagonists will enhance its toxicity towards non-cancer cells. In an effort to address this question, our lab has recently demonstrated that the combination does not synergize to kill peripheral blood mononuclear cells obtained from normal healthy donors [104]. Further work must be done to ensure that these potent combinations will maintain favorable tolerability when administered to patients.

mTOR inhibition as an adjuvant

Targeting oncogenic drivers

In contrast to targeting resistance mechanisms, others have found that combining oncogene-targeted therapies with mTOR inhibition also holds promise in B cell malignancies. For example, in Ph+ B-ALL driven by the BCR-ABL translocation, both rapamycin and PP242 strongly synergized with imatinib to suppress leukemia growth [99]. Similarly, in myeloproliferative disorders characterized by JAK2 mutations, combinations of TOR-KIs or rapalogs with JAK2 inhibitors synergistically killed cells whereas single-agent treatments were

primarily cytostatic [129,130]. In the activated B cell like (ABC) subtype of DLBCL, which is driven by sustained activation of the B cell receptor (BCR) [34], inhibition of the downstream kinase, Bruton's tyrosine kinase (BTK), also synergized strongly with PI3K/AKT/mTOR inhibitors [131]. However, the limitations of this approach are also becoming apparent. In particular, the germinal center B cell-like (GCB) DLBCL subtype is unresponsive to combinations of BTK and mTOR inhibitors likely because BCR activation is not an oncogenic driver in this setting [132]. More alarmingly, in some cases the addition of mTOR inhibitors may antagonize the effects of other agents either through suppression of proliferation or through induction of autophagy [133,134]. Studies like these serve as powerful reminders that a sound biological understanding supporting the use of these combinations must precede their clinical use.

Targeting histone deactylases (HDACs)

HDAC inhibitors are another promising class of drug that may benefit from the addition of mTOR inhibitors. In addition to modulating histone function and gene expression, HDACs also regulate the activity of non-histone proteins with relevance to B cell cancers (e.g. STAT, Hsp90, and FOXO) [135-138]. Importantly, mutations in genes regulating protein acetylation have been described in both B-ALL and NHL. For example, mutations in the CREBBP histone acetyltransferase (HAT) domain have been identified in a subset of patients with relapsed pediatric B-ALL where it may confer glucocorticoid resistance [139]. Similar mutations in HAT activity were identified as frequent mutations in both FL and DLBCL where their inactivation promotes aberrant up-regulation of BCL-6, a protein known to promote B cell malignancies [140-142]. Given the pervasive importance of protein acetylation, it is unsurprising that HDAC inhibitors have elicited promising responses in various leukemias and lymphomas. For example, in lymphomas with a t(14;18) translocation, HDAC inhibitors were shown to markedly reduce expression of BCL-2 leading to apoptosis [143]. In other contexts, HDAC inhibition can induce mitochondrial apoptosis via epigenetic regulation of other BCL-2 family proteins [144,145],

production of reactive oxygen species and ceramide [146], or activation of death receptors [147]. Potent anti-proliferative effects have also been described [145,148]. Importantly, recent evidence has suggested that the addition of mTOR inhibition may augment the effects of HDAC inhibitors. For example, our lab has recently identified synergy between HDAC inhibitors and TOR-KIs in B-ALL cell lines and primary patient samples [103]. Also, both temsirolimus and everlomius have demonstrated synergistic anti-proliferative and apoptotic effects when combined with the HDAC inhibitors in MCL [149,150]. In DLBCL, combining HDAC inhibitors with rapalogs or TOR-KIs also synergistically induced apoptosis [65,151]. While there is still debate as to the exact mechanism of synergy, it is clear that in a preclinical setting this combination has marked potential in B cell malignancies. However, in a phase I trial combining panobinostat and everolimus in relapsed/refractory lymphoma, the combination yielded ORRs similar to everolimus alone but with higher incidence of thrombocytopenia [152]. As this combination moves forward, it will be important to identify the exact mechanism of action so as to better predict which patients may benefit from these combinations. It may also be useful to explore compounds targeting selected subsets of cellular HDAC enzymes.

Targeting the proteasome

Another class of inhibitors that has shown promise in B cell malignancies are proteasome inhibitors [153]. Interestingly, even across several cancer subtypes these inhibitors have been most promising in B cell malignancies [154-159] as evidenced by FDA approval for bortezomib in both relapsed MCL and multiple myeloma [160]. By suppressing degradation of proteins, these inhibitors induce a plethora of cellular responses leading to anti-proliferative and pro-apoptotic effects [161,162]. Most notable among these effects are its ability to suppress NF-kB activity and modulate expression of BCL-2 family proteins [162-164], which provides the basis for single agent bortezomib efficacy in ABC-DLBCL [165,166]. However, in other B cell malignancies, single agent proteasome inhibition is not as effective [167-169]. While preclinical

data has suggested some synergy between rapalogs and bortezomib [150,170], whether combined proteasome and mTOR inhibition will have generalizable efficacy is still unclear. A major clinical concern with bortezomib is neurological toxicity [171,172], and while dose management may alleviate some risks, it is unclear what effects the addition of mTOR inhibitors may have on patient outcomes.

Outlook

While the initial discovery of mTOR inhibitors yielded a flood of promising and exciting preclinical data, the initial wave of rapamycin-based therapies have not elicited widespread and durable patient responses. Consequently, rapalogs have only achieved regulatory approval in one subtype. With the development of TOR-KIs that offered a distinct biochemical advantage over rapalogs, there was an expectation of much greater responses. While the clinical data are not yet mature, it is becoming more apparent that while TOR-KIs may indeed have higher efficacy, it comes with the cost of higher toxicities. Whether dose modifications or altered schedules can lower the toxicity while maintaining efficacy is still unknown, but is a critical question in determining the future of mTOR-targeted therapies. Given the modest performance of single-agent mTOR inhibitors, it is likely that identifying combinations, either with targeted agents or with chemotherapy, may be the key to unleashing the full potential of mTOR inhibition in cancer. While the preclinical data strongly support this claim, it is still unclear whether this approach will translate to improved clinical responses, and more importantly, whether it will do so with acceptable toxicities. Given the generally well-tolerated nature of rapalogs, it seems prudent to initiate these combination studies using rapalogs. It will also be important to emphasize the preclinical evaluation of cancer selectivity, specifically to address whether these combinations will synergize to kill normal cells. Thus, the field of mTOR targeted therapies has progressed rapidly over the past few decades, and as our knowledge of the biology increases,

so too will our capacity to augment and fine-tune these therapies to effect positive patient outcomes.

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Conflict of Interest Statement

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years. D.A.F. reports a patent, mTOR modulators and uses thereof, licensed to Intellikine, Inc.

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Figure Legends

Figure 1: mTOR signaling pathway

mTOR exists in two distinct complexes (mTORC1 and mTORC2) that are regulated separately and have distinct substrates. Whereas mTORC2 is regulated downstream of PI3K, mTORC1 is coordinately regulated by growth factor signals, nutrient availability (amino acids), and cellular energy status (ATP levels). The outputs of their downstream effectors coordinate processes required for cell growth including survival, inhibition of autophagy, protein translation and cell cycle progression.

Figure 2: Mechanisms of aberrant mTOR activation in B-cell malignancies

Different proteins are amplified or activated (shown in red) in B-cell malignancies that result in increased mTOR activity. Where as the loss of the tumor suppressor PTEN (shown in blue) promotes mTOR activation. These mutations negate the normal constraints on mTOR activity to promote cancer cell proliferation.

Figure 3: Different effects of rapamycin and TOR-KI on mTOR activity

Rapamycin (and all rapalogs) only partially inhibits mTOR activity. Rapamycin forms a complex with FKB12 to inhibit TORC1 activation of S6K activity and only partially reduces effects on 4EBP. S6K normally negatively feeds back to inhibit the activation of PI3K. By suppressing S6K, rapamycin negates the feedback inhibition resulting in increased PI3K, TORC2 and AKT activation. Thus, survival signals from AKT highly active and 4EBP is partially active. Conversely, TOR-KIs suppresses all mTOR survival outputs.

Figure 4: Combination of targeting BCL-2 and mTOR

(A) The BCL-2 family of proteins consists of pro- and anti-apoptotic members that interact antagonistically to determine cell fate. Survival signaling through AKT and mTOR increases the

anti-apoptotic family members and decreases the pro-apoptotic members. (B) By inhibiting mTOR activity the balance is shifted where anti-apoptotic family members are reduced and pro-apoptotic members are increased. This reduces the capacity of anti-apoptotic proteins to sequester the pro-apoptotic proteins. Addition of ABT-199, a BCL-2 inhibitor, further shifts this balance to release the pro-apoptotic proteins and cause cancer killing.

Targeting mTOR for the treatment of B cell malignancies

J. Scott Lee, Thanh-Trang Vo, and David A. Fruman

Department of Molecular Biology & Biochemistry, University of California, Irvine

Corresponding Author:

David A. Fruman, PhD

3242 McGaugh Hall

University of California, Irvine

Irvine, CA 92697

Tel: (949) 824-1947

Fax: (949) 824-8551

Email: dfruman@uci.edu

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~~Summary~~Abstract

Mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulator of cell growth, division, and survival. Many hematologic malignancies exhibit elevated or aberrant mTOR activation, supporting the launch of numerous clinical trials aimed at evaluating the potential of single-agent mTOR-targeted therapies. While promising early clinical data using allosteric mTOR inhibitors (rapamycin and its derivatives, rapalogs) have suggested activity in a subset of hematologic malignancies, these agents have shown limited efficacy in most contexts. Whether the efficacy of these partial mTOR inhibitors might be enhanced by more complete target inhibition is being actively addressed with second generation ATP-competitive mTOR kinase inhibitors (TOR-KIs), which have only recently entered clinical trials. However, emerging preclinical data suggest that despite their biochemical advantage over rapalogs, TOR-KIs may retain a primarily cytostatic response. Rather, combinations of mTOR inhibition with other targeted therapies have demonstrated promising efficacy in several preclinical models. This review investigates the current status of rapalogs and TOR-KIs in B cell malignancies, with an emphasis on emerging preclinical evidence of synergistic combinations involving mTOR inhibition.

Introduction

The mTOR Signaling Pathway

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mTOR is a serine/threonine kinase that functions as a master regulator of cell growth, proliferation, metabolism, and survival. mTOR is active in two distinct multi-protein complexes (mTORC1 and mTORC2) that are characterized by the defining subunits RAPTOR and RICTOR respectively [1,2]. Each complex is differentially regulated and has a distinct set of substrates (Figure 1).- Activation of mTORC2 ~~activation~~ is incompletely understood, but has recently been shown to be ~~directly~~ dependent on the generation of~~regulated by the levels of~~ PI(3,4,5)P_3 ~~produced~~ by phosphoinositide 3-kinase (PI3K) [3]. Upon activation mTORC2

functions to amplify the activity of AKT, ~~,~~a key oncogene involved in cell survival and metabolism [4,5]~~ and is required for full activity of AKT [4], a key oncogene involved in cell survival and metabolism [5]~~. On the other hand, mTORC1 activation is coordinately regulated by ~~functions by integrating~~ growth factor signals (i.e. from the PI3K/AKT pathway), ~~and~~ nutrient availability (amino acids), and cellular energy status (ATP levels). Under conditions of low nutrients, amino acid sensors (such as SLC38A9 [6,7]) suppress mTORC1 activation. Similarly, under conditions of low energy (low ATP), 5' AMP-activated protein kinase (AMPK) can also suppress mTORC1 activation [8]. This multifaceted regulation ~~to~~ ensures that the cell is at an appropriate bioenergetic state to support cell growth and division [9,10]~~[6,7]~~ (Figure 1).

Upon activation, mTORC1 promotes key biosynthetic pathways including translation, transcription, and lipogenesis, while suppressing apoptotic and autophagic processes [11,12]~~[8,9]~~. The most well-characterized downstream targets of mTORC1 ~~include~~ are the p70 ribosomal-S6 kinases (S6Ks)~~, which are critical for lipid and ribosome biogenesis pathways,~~ and eukaryotic initiation factor 4E (eIF4E) binding proteins (4E-BPs)~~.~~ Phosphorylation of S6Ks induces its activity, which is critical for lipid and ribosome biogenesis pathways and promotes translation via suppression of PDCD4 and activation of eIF4B [13,14]. In contrast, phosphorylation of 4E-BPs suppresses their ability to inhibit eIF4E, which promotes translation initiation~~which promotes translation of cap-bound mRNA transcripts (Figure 1). Whereas mTORC1 activates S6Ks directly, it activates eIF4E indirectly by suppressing the inhibitory function of eIF4E binding proteins (4E-BPs)~~ [15]~~[10]~~. Together, these effectors ~~promote~~ coordinately increase~~d~~ protein synthesis rates, a process whose dysregulation is a central driving mechanism in cancer [16,17]~~[11,12]~~. Importantly, hyper-activating mutations in mTOR itself have been identified in many cancers and~~s,~~ further indicate~~s~~~~ing~~ the importance of mTOR activity to tumorigenesis [18]~~[13]~~.

Evidence of mTOR activation in B-ALL and NHL

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Aberrant activation of mTOR is frequently associated with poorer prognosis and has been well described in B cell malignancies including B cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkin's lymphoma (NHL). Given that mTOR is a convergence point for many distinct signaling pathways, there are many mechanisms by which it may become inappropriately activated (Figure 2). In B-ALL, the most common mode is through activation of upstream kinases. For example, the Philadelphia chromosome (Ph+), characterized by the BCR-ABL translocation, induces robust activation of several parallel pathways leading to mTOR activation. Similarly, genomic profiling has recently identified a Ph-like subset of B-ALL, which exhibits a similar kinase activation signature to that of Ph+ B-ALL. Notably, these mutations are strongly associated with poorer outcomes in both children and adults [19-22]~~[14-17]~~. Empirical evidence has also shown a direct correlation between AKT and/or mTOR activation and poor prognosis in patients with pediatric and adult B-ALL [23-25]~~[18-20]~~.

Among NHL subtypes, activation of mTOR is consistently a reliable indicator of more aggressive disease and poorer prognosis [26-30]~~[21-25]~~. Similar to B-ALL, activation of mTOR follows through direct mutations in key upstream pathways. In mantle cell lymphoma (MCL), amplification of *PIK3CA* (the gene encoding the catalytic subunit of PI3K) and/or PTEN loss (the negative regulator of PI3K activity) have been observed in a large fraction of primary tissue samples [31]~~[26]~~. In diffuse large B cell lymphoma (DLBCL), activation may be similarly achieved via mutations in *PIK3CA* [32,33]~~[27,28],~~ or chronic B cell receptor activation [34]~~[29]~~. In follicular lymphoma (FL), mTOR is aberrantly activated by way of PKCζ or Syk kinases [35-38]~~[30-33]~~. Collectively, these data highlight the impact of elevated mTOR activity on patient outcomes, and provide a solid rationale for the use of mTOR-targeted therapies in these B cell malignancies.

~~Rapalogs~~Rapamycin and Rapalogs: partial mTORC1 inhibitors

Mechanism of action

Upon entry into a cell, rapamycin binds to FKBP12~~,~~ forming a complex that potently and selectively suppresses mTORC1 kinase activity by limiting substrate access to the active site [39,40][~~34,35~~]. Importantly, the rapamycin-FKBP12 complex cannot bind to mTORC2 [2,41][~~2,36~~], though in some cases~~,~~ prolonged exposure may limit the assembly of mTORC2 [42][~~37~~]. In this manner, rapamycin behaves as a highly potent and selective inhibitor of mTORC1 (Figure 3). However, poor solubility and pharmacokinetics spurred the development of rapamycin analogs (termed rapalogs) for oral dosing in cancer patients [43][~~38~~]. Most notable among these rapalogs are temsirolimus (CCI-779, Wyeth Pharmaceuticals [44][~~39~~]), everolimus (RAD001, Novartis Pharmaceuticals [45][~~40~~]), and ridaforolimus (AP23573, Merck and ARIAD Pharmaceuticals [46][~~41~~]).

Rapamycin and Rapalogs in B-ALL

Early testing with rapamycin unveiled potent anti-proliferative efficacy in several preclinical models of ALL. In an Eμ-RET model of murine B-pre ALL, rapamycin as a single agent potently inhibited proliferation of leukemia cells both *in vitro* and *in vivo* [47,48][~~42,43~~]. Similar efficacy was later observed in models of Ph+ B-ALL [49,50][~~44,45~~]~~,~~ as well as in Ph-like B-ALL~~,~~ driven by JAK pathway mutations or CRLF2 rearrangement [51][~~46~~]. Rapalogs also demonstrated marked preclinical efficacy in primary human ALL samples grown *in vitro* or in xenograft models [50-52]. Notably, rapamycin demonstrated single-agent cytotoxicity in primary pediatric ALL samples~~,~~ and sensitized cells to doxorubicin *in vitro* [52][~~47~~]. Both everolimus and temsirolimus have shown similar efficacy in xenograft models of adult and pediatric primary human ALL as single agents [53][~~48~~] and in combination with chemotherapy [54,55][~~49,50~~].

Clinically, rapamycin as a single agent exhibited no dose-limiting toxicities, but had lackluster efficacy compared to standard chemotherapeutic options (Table 1). In an early trial, rapamycin yielded stable disease in only three out of nine pediatric patients with relapsed ALL [56][~~51~~]. As a result, several trials have been launched to determine whether rapalogs can

combine safely and effectively with standard chemotherapies. An early pilot trial combining rapamycin with glucocorticoids in relapsed ALL patients found that rapamycin effectively reduced the anti-apopotic protein MCL-1 in various patients. This promising outcome suggested that rapamycin might sensitize ALL cells to apoptosis-inducing drugs. Indeed, in another study combining temsirolimus with intensive multi-drug re-induction therapy (dexamethasone, mitoxantrone, vincristine, and PEG-asparaginase) in relapsed childhood ALL yielded complete response in seven of sixteen patients, of which three had less than 0.01% minimal residual disease (MRD) by the end of treatment [57]~~[52]~~. However, a separate trial evaluating everolimus combined with intensive chemotherapy (hyper-CVAD) in relapsed B-ALL yielded complete remission rates that were similar to standard salvage chemotherapies (~35%) [58-60]~~[53-55]~~. These trials highlight how the efficacy of rapalogs seem to be dependent on which chemotherapeutics are used, warranting further investigation.

A key question that remains to be answered is whether rapalogs combined with chemotherapy will demonstrate acceptable toxicity profiles. In the aforementioned trial combining temsirolimus with re-induction chemotherapy, the treatment was associated with unacceptable toxicities including severe infections that led to one death due to sepsis [57]~~[52]~~. However, a recent multi-center study testing the combination of everolimus with prednisone, vincristine, PEG-asparaginase and doxorubicin demonstrated that the combination was well tolerated in pediatric patients with first relapse [61]. Further trials are being performed, including an expansion of the aforementioned trial as well as ~~As a result, a trial~~one testing the safety of temsirolimus with less intensive re-induction ~~with~~ (etoposide and cyclophosphamide ~~is currently underway :~~ (NCT01614197). ~~Additionally, a multi-center study is also testing the combination of everolimus with prednisone, vincristine, PEG-asparaginase and doxorubicin (NCT01523977).~~ Together, these results show that rapalogs have some potential in combination therapy, but an effective and tolerable regimen in B-ALL has yet to be identified. Moving forward, it will be

important to identify which chemotherapeutics are best combined with rapalogs and whether modifications to the dose and/or schedule may alleviate dose-limiting toxicities.

Rapalogs in NHL

Similar to B-ALL, preclinical testing of rapalogs in NHL revealed promising cytostatic effects both *in vitro* and *in vivo*, yet clinical responses were limited in most contexts. For example, in MCL, FL, and DLBCL, rapamycin potently suppressed the proliferation of cell lines and primary patient cells *in vitro* [62-66]~~[56-60]~~. However, the clinical use of rapalogs has only made progress in MCL where ~~chemotherapeutic~~ responses to standard chemotherapies are limited (Table 1). In phase II trials of relapsed MCL, single agent administration of either temsirolimus or ridaforolimus yielded overall response rates (ORR) of 38% [67]~~[61]~~ and 33% [68]~~[62]~~ respectively. Notably, a subsequent phase II trial using a 10-fold lower dose of temsirolimus revealed that similar responses could be obtained with lower toxicity [69]~~[63]~~. Based on these results, a randomized phase III trial was conducted. Strikingly, the ~~overall response rates~~ORR and progression free survival were significantly higher in patients treated with temsirolimus compared to investigator's choice agent. These results ultimately led to approval for temsirolimus as a single agent therapy for relapsed/refractory MCL in Europe [70]~~[64]~~. A subsequent phase II trial has also been completed combining temsirolimus with rituximab in relapsed/refractory MCL. Despite demonstrating higher response rates than single agent temsirolimus, the combination was also associated with higher toxicities including thrombocytopenia and neutropenia in a significant fraction of patients [71]~~[65]~~. Rapalog monotherapy has also elicited responses in a subset of patients with other NHL subtypes. In a phase II trial of everolimus in relapsed lymphoma, the ORR in DLBCL was 30% (14/47) and 38% (3/8) in FL [72]~~[66]~~. Similar results were seen with temsirolimus where the ORR was 28% for DLBCL and 53% in FL [73]~~[67]~~. While these studies highlight that rapalogs have some activity, the availability of better therapeutic options in both DLBCL and FL has limited the

clinical progress of rapalogs in these diseases. Thus, across NHL subtypes it will be important to determine whether the addition of rapaogs to standard chemotherapy can provide additional benefit to patients, without increasing toxicities.

Outlook:

Overall, despite showing promising preclinical activity in hematologic malignancies, rapalogs have only gained regulatory approval for use in one disease setting (MCL) where standard chemotherapies have limited efficacy. A major issue is that rapalogs given as single agents tend to elicit primarily cytostatic responses in hematologic malignancies [62,63,66,74][~~56,57,60,68~~]. Clinically, the lack of inherent cytotoxicity is problematic since discontinuation of treatment may permit tumor cell regrowth [75-77][~~69-71~~]. While continued treatment may combat this issue, whether rapalogs at anti-leukemic doses will be safe for long-term use also remains to be seen. Clinical evidence of several toxicities including thrombocytopenia, mucositis, and hyperlipidemia suggests that prolonged treatment will be difficult to manage [43][~~38~~]. Alternatively, combinations with chemotherapy are actively being investigated and may reposition rapalogs as an adjuvant to improve chemotherapeutic responses. On this note, it is important to point out that the cytostatic activity of rapalogs will likely limit its potential to combine with certain chemotherapies, necessitating the identification of cytotoxic drugs that will synergize with rapalogs productively while maintaining acceptable tolerability.

While rapalogs provided proof-of-concept for effective mTOR targeted anti-cancer therapies, they exhibit many unfavorable biochemical properties that may also limit their clinical potential. Most notably, failure to suppress mTORC2 kinase activity allows maintained survival signaling through AKT and other related kinases. This issue is exacerbated by the existence of a negative feedback loop downstream of mTORC1 (Figure 3). Selective inhibition of mTORC1 induces robust feedback activation of upstream PI3K/AKT and MAPK pathways~~,~~ allowing cancer cells to escape from the effects of rapamycin [57,78-82][~~52,72-76~~]. Additionally, rapalogs are known to

incompletely inhibit the phosphorylation of a subset of mTORC1 substrates (Figure 3). Despite restricting access to the active-site, rapalog-induced suppression of 4E-BP1 phosphorylation is refractory to long-term treatment compared to phosphorylation of p70S6K [83][~~77~~]. The cause of this differential sensitivity has recently been attributed to distinct substrate sequences near the phosphorylation sites [84][~~78~~]. This incomplete suppression of mTORC1 may significantly impact the anti-cancer potential of rapalogs as sustained activation of eIF4E is known to promote oncogenesis [85][~~79~~]. Consequently, sustained 4E-BP phosphorylation may allow cancer cells to escape from rapamycin-induced cell cycle arrest [86][~~80~~]. Thus, more complete mTOR inhibition may be required to elicit more promising clinical responses.

TOR-KIs: complete mTORC1/2 inhibitors

The timely development of mTOR kinase inhibitors (TOR-KIs) directly addressed the biochemical disadvantages of rapalogs. By competing with ATP for binding to the mTOR active site, not only do TOR-KIs more completely block mTORC1 substrate phosphorylation (namely 4E-BPs), but they also inhibit mTORC2 activity [87,88][~~81,82~~]. This results in reduced phosphorylation of AKT at Ser473 (Figure 3), dampening the feedback activation of PI3K/AKT that is known to limit rapalog efficacy [89-91][~~83-85~~]. It is important to note that by competing with ATP, TOR-KIs are capable of inhibiting several kinases at higher doses, including the structurally related protein, PI3K. Conversely, several compounds that are often used pre-clinically as PI3K inhibitors (wortmannin, LY294002) directly inhibit mTORC1 and mTORC2 at similar concentrations. Thus, it is important to fully understand the pharmacologic properties of ATP-competitive mTOR and PI3K inhibitors when interpreting their preclinical and clinical efficacy.

Several structurally distinct mTOR-selective inhibitors have been reported and tested in models of B cell malignancies. Most notable among them are PP242 [88][~~82~~], Torin1 [87][~~81~~], Ku-0063794 [92][~~86~~], AZD8055 [93][~~87~~], AZD2014 [93][~~87~~], MLN0128 (previously INK128

[94][88]), and CC-223 [95][89]. In preclinical testing, these TOR-KIs proved superior to rapalogs in terms of cytostatic and cytotoxic potential. For example, in a mouse model of AKT-driven lymphangiogenesis, PP242 strongly suppressed both 4E-BP1 phosphorylation and tumor growth compared to rapamycin [96][90]. These findings were also recapitulated *in vitro* using leukemia and DLBCL cell lines where TOR-KIs had a greatly improved biochemical effect on downstream 4E-BP phosphorylation [97-99][91-93].

Despite the broader biochemical impact of TOR-KIs over rapalogs, whether complete mTOR kinase inhibition is sufficient to elicit cytotoxic responses is yet to be established. Two reports of structurally distinct TOR-KIs in B-ALL demonstrated that mTOR kinase inhibition was sufficient to induce apoptosis in B-ALL cell lines compared to rapamycin [100,101][94,95]. However, in both studies, apoptosis was only observed at doses of TOR-KI that greatly exceed what was needed to fully suppress mTOR kinase activity as measured by western blot. At lower doses that still fully suppress mTOR activity, our lab has found that both AZD8055 and MLN0128 maintain a primarily cytostatic response profile (that is greater than rapalogs) [98,102-104][92,96-98]. Notably, low doses of PP242 were sufficient to kill murine bone marrow cells immortalized by p190-BCR-ABL [99][93], suggesting that fully transformed B-ALL cells with additional oncogenic lesions may respond differently to mTOR inhibition. Thus, it remains unclear whether TOR-KIs will be effective in B-ALL or NHL as a single agents at doses that are highly selective for mTOR kinase activity.

Early clinical trials have suggested that while TOR-KIs are more effective than rapalogs at suppressing tumor growth, they may also be less tolerable [78][72]. A single agent tolerability test of AZD2014 showed dose-limiting toxicities that were similar to rapalogs including mucositis and fatigue [105][99]. Both CC-223 and MLN0128~~,~~ also presented similar toxicities, but ~~in addition to~~ hyperglycemia~~,~~ also occurred and necessitated~~ting~~ close monitoring of patient blood [106,107][100,101]. Several additional clinical trials are currently in progress to address the efficacy and tolerability of TOR-KIs and are summarized in **Table 2**. However, a key question is

to investigate whether TOR-KIs will retain anti-cancer efficacy at lower doses that minimize these toxicities. While it is likely that lowering the dose of TOR-KIs may improve their tolerability, it will also impinge on their ability to fully suppress mTOR kinase activity. Moving forward, it may be important to determine whether these potentially suboptimal doses, which only partially inhibit mTOR, will be more effective than clinically tolerable doses of rapalogs, which potently inhibits phosphorylation of some, but not all, mTORC1 substrates.

Emerging Combinations with mTOR Inhibitors:

Recent research efforts have been dedicated to identifying promising combinations that can synergistically kill cancer cells. The rationales behind these emerging combinations can be loosely categorized into two broad groups. The first approach seeks to exploit known resistance mechanisms to mTOR inhibition; either by targeting feedback pathways or using apoptosis-sensitizing agents. The second approach seeks to evaluate the potential of mTOR inhibitors as adjuvants to augment the effects of other agents targeting known oncogenic drivers. While both approaches have yielded several promising combinations, whether they can be translated to significant clinical responses with acceptable toxicity still remains to be determined.

Combinations targeting resistance mechanisms

Targeting parallel and downstream pathways

As with all targeted therapies, an understanding of how cells maintain survival in the presence of mTOR inhibitors has been crucial to the identification of promising combinations. Currently, there are several known acquired and *de novo* mechanisms of resistance to mTOR-targeted therapies. For example, in addition to feedback activation of PI3K/AKT, mTORC1 inhibition may also activate the parallel MAPK/ERK pathway in B-ALL ~~(Figure 4)~~. In a similar fashion, PI3K/AKT/mTOR inhibition can also induce up-regulation of receptor tyrosine kinases (RTKs) leading to resistance in some solid tumors [108]~~[102]~~. In agreement with these induced

resistance mechanisms, the addition of MAPK inhibitors and RTK inhibitors have demonstrated significantly more efficacy in combination with both rapalogs and TOR-KIs in preclinical settings [80,109,110][~~74,103,104~~]. However, in other instances~~,~~ resistance to mTOR inhibition may be a result of sustained downstream effector activity, particularly cap-dependent translation. For example, our laboratory has noted resistance to TOR-KIs in DLBCL cell lines lacking expression of 4E-BPs [98][~~92~~] or ~~, and~~ over-expressi~~o~~n~~g of~~ eIF4E ~~limits the efficacy of TOR-KIs~~ [111][~~105~~]. Furthermore, recent evidence has indicated that PIM and MNK kinases can maintain cap-dependent translation downstream of mTORC1 inhibition [112][~~106~~] ~~(Figure 4)~~. In these situations, targeting cap-dependent translation indirectly using combinations of PIM or MNK inhibitors with TOR-KIs has shown cytotoxic activity in AML cell lines [113,114][~~107,108~~] as well as in cutaneous T cell lymphoma cell lines *in vitro* [115][~~109~~]. Additional work is required to evaluate the potential of directly targeting the cap-dependent translation initiation machinery. It is likely that other mechanisms of resistance will arise as our experience with mTOR inhibitors increases, and these may ultimately support the study of additional combinations.

While clinical data regarding the efficacy of these combinations in B cell malignancies has not reached maturity, similar combinations have been successfully deployed in non-hematologic malignancies. For example, inhibition of the upstream tyrosine kinase, HER2, significantly improved the efficacy of mTORC1/2 inhibition in patients with refractory breast cancer compared to single agent treatment [116][~~110~~]. Similarly, combinations of PI3K/AKT/mTOR and Ras/MAPK/ERK pathway inhibition yielded improved response rates in patients with advanced refractory solid tumors, but did so at the cost of significantly higher toxicities [117][~~111~~]. Collectively these studies highlight the potential of using mTOR inhibitors in combination with agents targeting known resistance pathways to mTOR inhibition~~,~~ or as an adjuvant therapy to augment the effects of other rational targeted therapies. However, it will be important to determine whether these combinations targeting multiple key survival pathways will remain selective for cancer cells as toxicity will be a major concern.

Targeting apoptosis

Another straightforward approach to directly enhance~~ing~~ the apoptotic potential of mTOR inhibition is to target the pro-survival BCL-2 family proteins. Apoptosis is regulated through dynamic and competitive binding interactions between anti-apoptotic proteins (e.g. BCL-2, BCL-XL, BCL-w, and MCL-1) and pro-apoptotic sensitizers (e.g. BAD, PUMA, and NOXA), activators (e.g. BIM and BID), and effectors (BAX and BAK) (Figure ~~45~~A). While mTOR inhibition is known to suppress survival signaling through both mTORC1 (e.g. MCL-1 expression [96]~~[90]~~) and AKT (e.g. inhibition of BAD and down-regulation of BIM [118,119]~~[112,113]~~), TOR-KIs are insufficient to induce apoptosis through this pathway. Thus, a simple approach would be to use antagonists of the pro-survival proteins to disrupt their binding capacity, and subsequently lower the threshold for BIM to activate BAX/BAK-mediated MOMP and apoptosis [120]~~[114]~~.

ABT-737, and its orally bioavailable analog, ABT-263, represent the most potent and selective small molecule inhibitors of BCL-2 and BCL-X_L. Both of these compounds demonstrated remarkable cytotoxic potential that was significantly enhanced when combined with mTOR inhibitors in DLBCL [121]~~[115]~~, FL [122]~~[116]~~, AML [123]~~[117]~~, and B-ALL [124]~~[118]~~. However, due to on-target toxicity associated with BCL-X_L inhibition [125]~~[119]~~, a more promising clinical candidate is ABT-199 [126]~~[120]~~. ABT-199 is a selective inhibitor of BCL-2~~,~~ and has elicited substantial clinical responses in patients with CLL as a single agent [127]~~[121]~~, leading to its designation as a breakthrough therapy for CLL patients with a 17p deletion (p53). Importantly, we and others have recently reported that ABT-199 synergizes with mTOR inhibition comparably to dual BCL-2/BCL-X_L inhibitors [104,128]~~[98,122]~~, suggesting that the rationale established using first generation BCL-2 antagonists will hold true for ABT-199. However, a key concern is whether the addition of TOR-KIs to BCL-2 antagonists will enhance its toxicity towards non-cancer cells. In an effort to address this question, our lab has recently demonstrated that the combination does not synergize to kill peripheral blood mononuclear cells

obtained from normal healthy donors [104][~~98~~]. Further work must be done to ensure that these potent combinations will maintain favorable tolerability when administered to patients.

mTOR inhibition as an adjuvant

Targeting oncogenic drivers

In contrast to targeting resistance mechanisms, others have found that combining oncogene-targeted therapies with mTOR inhibition also holds promise in B cell malignancies. For example, in Ph+ B-ALL driven by the BCR-ABL translocation, both rapamycin and PP242 strongly synergized with imatinib to suppress leukemia growth [99][~~93~~]. Similarly, in myeloproliferative disorders characterized by JAK2 mutations, combinations of TOR-KIs or rapalogs with JAK2 inhibitors synergistically killed cells whereas single-agent treatments were primarily cytostatic [129,130][~~123,124~~]. In the activated B cell like (ABC) subtype of DLBCL, which is driven by sustained activation of the B cell receptor (BCR) [34][~~29~~], inhibition of the downstream kinase, Bruton's tyrosine kinase (BTK), also synergized strongly with PI3K/AKT/mTOR inhibitors [131][~~125~~]. However, the limitations of this approach are also ~~recently~~ becoming apparent. In particular, the germinal center B cell-like (GCB) DLBCL subtype is unresponsive to combinations of BTK and mTOR inhibitors~~,~~ likely because BCR activation is not an oncogenic driver in this setting [132][~~126~~]. More alarmingly, in some cases the addition of mTOR inhibitors may antagonize the effects of other agents~~,~~ either through suppression of proliferation or through induction of autophagy [133,134][~~127,128~~]. Studies like these serve as powerful reminders that a sound biological understanding supporting the use of these combinations must precede their clinical use.

Targeting histone deactylases (HDACs)

HDAC inhibitors are another promising class of drug that may benefit from the addition of mTOR inhibitors. In addition to modulating histone function and gene expression, HDACs also regulate the activity of non-histone proteins with relevance to B cell cancers (e.g. STAT, Hsp90, and FOXO) [135-138][~~129-132~~]. Importantly, mutations in genes regulating protein acetylation have been described in both B-ALL and NHL. For example, mutations in the CREBBP histone acetyltransferase (HAT) domain have been identified in a subset of patients with relapsed pediatric B-ALL where it may confer glucocorticoid resistance [139][~~133~~]. Similar mutations in HAT activity were identified as frequent mutations in both FL and DLBCL where their inactivation promotes aberrant up-regulation of BCL-6, a protein known to promote B cell malignancies [140-142][~~134-136~~]. Given the pervasive importance of protein acetylation, it is unsurprising that HDAC inhibitors have elicited promising responses in various leukemias and lymphomas. For example, in lymphomas with a t(14;18) translocation, HDAC inhibitors were shown to markedly reduce expression of BCL-2 leading to apoptosis [143][~~137~~]. In other contexts, HDAC inhibition can induce mitochondrial apoptosis via epigenetic regulation of other BCL-2 family proteins [144,145][~~138,139~~], production of reactive oxygen species and ceramide [146][~~140~~], or activation of death receptors [147][~~141~~]. Potent anti-proliferative effects have also been described [145,148][~~139,142~~]. Importantly, recent evidence has suggested that the addition of mTOR inhibition may augment the effects of HDAC inhibitors. For example, our lab has recently identified synergy between HDAC inhibitors and TOR-KIs in B-ALL cell lines and primary patient samples [103][~~97~~]. Also, both temsirolimus and everlomius have demonstrated synergistic anti-proliferative and apoptotic effects when combined with the HDAC inhibitors in MCL [149,150][~~143,144~~]. In DLBCL, combining HDAC inhibitors with rapalogs or TOR-KIs also synergistically induced apoptosis [65,151][~~59,145~~]. While there is still debate as to the exact mechanism of synergy, it is clear that in a preclinical setting~~,~~ this combination has marked potential in B cell malignancies. However, in a phase I trial combining panobinostat and everolimus in relapsed/refractory lymphoma, the combination yielded ORRs similar to

everolimus alone~~,~~ but with higher incidence of thrombocytopenia [152][~~146~~]. As this combination moves forward, it will be important to identify the exact mechanism of action so as to better predict which patients may benefit from these combinations. It may also be useful to explore compounds targeting selected subsets of cellular HDAC enzymes.

Targeting the proteasome

Another class of inhibitors that has shown promise in B cell malignancies are proteasome inhibitors [153][~~147~~]. Interestingly, even across several cancer subtypes~~,~~ these inhibitors have been most promising in B cell malignancies [154-159][~~148-153~~]~~,~~ as evidenced by ~~the~~ FDA approval for bortezomib in both relapsed MCL and multiple myeloma [160][~~154~~]. By suppressing degradation of proteins, these inhibitors induce a plethora of cellular responses leading to anti-proliferative and pro-apoptotic effects [161,162][~~155,156~~]. Most notable among these effects are its ability to suppress NF-kB activity and modulate expression of BCL-2 family proteins [162-164][~~156-158~~], which provides the basis for single agent bortezomib efficacy in ABC-DLBCL [165,166][~~159,160~~]. However, in other B cell malignancies, single agent proteasome inhibition is not as effective [167-169][~~161-163~~]. While preclinical data has suggested some synergy between rapalogs and bortezomib [150,170][~~144,164~~], whether combined proteasome and mTOR inhibition will have generalizable efficacy is still unclear. A major clinical concern with bortezomib is neurological toxicity [171,172][~~165,166~~], and while dose management may alleviate some risks, it is unclear what effects the addition of mTOR inhibitors may have on patient outcomes.

Outlook

While the initial discovery of mTOR inhibitors yielded a flood of promising and exciting preclinical data, the initial wave of rapamycin-based therapies have not elicited widespread and durable patient responses. Consequently, rapalogs have only achieved regulatory approval in

one subtype. With the development of TOR-KIs that offered a distinct biochemical advantage over rapalogs, there was an expectation of much greater responses. While the clinical data are not yet mature, it is becoming more apparent that while TOR-KIs may indeed have higher efficacy, it comes with the cost of higher toxicities. Whether dose modifications or altered schedules can lower the toxicity while maintaining efficacy is still unknown, but is a critical question in determining the future of mTOR-targeted therapies. Given the modest performance of single-agent mTOR inhibitors, it is likely that identifying combinations, either with targeted agents or with chemotherapy, may be the key to unleashing the full potential of mTOR inhibition in cancer. While the preclinical data strongly support this claim, it is still unclear whether this approach will translate to improved clinical responses, and more importantly, whether it will do so with acceptable toxicities. Given the generally well-tolerated nature of rapalogs, it seems prudent to initiate these combination studies using rapalogs. It will also be important to emphasize the preclinical evaluation of cancer selectivity, specifically to address whether these combinations will synergize to kill normal cells. Thus, the field of mTOR targeted therapies has progressed rapidly over the past few decades, and as our knowledge of the biology increases, so too will our capacity to augment and fine-tune these therapies to effect positive patient outcomes.

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Conflict of Interest Statement

All authors have completed the Unified Competing Interest form at

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http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years. D.A.F. reports a patent, mTOR modulators and uses thereof, licensed to Intellikine, Inc.

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Figure Legends

Figure 1: mTOR signaling pathway

mTOR exists in two distinct complexes (mTORC1 and mTORC2) that are regulated separately and have distinct substrates. Whereas mTORC2 is regulated downstream of PI3K, mTORC1 is coordinately regulated by growth factor signals, nutrient availability (amino acids), and cellular energy status (ATP levels). The outputs of their downstream effectors coordinate processes required for cell growth including survival, inhibition of autophagy, protein translation and cell cycle progression.

Figure 2: Mechanisms of aberrant mTOR activation in B-cell malignancies

Different proteins are amplified or activated (shown in red) in B-cell malignancies that result in increased mTOR activity. Where as the loss of the tumor suppressor PTEN (shown in blue) promotes mTOR activation. These mutations negate the normal constraints on mTOR activity to promote cancer cell proliferation.

Figure 3: Different effects of rapamycin and TOR-KI on mTOR activity

Rapamycin (and all rapalogs) only partially inhibits mTOR activity. Rapamycin forms a complex with FKB12 to inhibits TORC1 activation of S6K activity and only partially reduces effects on 4EBP. S6K normally negatively feeds back to inhibit the activation of PI3K. By suppressing S6K, rapamycin negates the feedback inhibition resulting in increased PI3K, TORC2 and AKT activation. Thus, survival signals from AKT highly active and 4EBP is partially active. Conversely, TOR-KIs suppresses all mTOR survival outputs.

Figure 4: Combination of targeting BCL-2 and mTOR

(A) The BCL-2 family of proteins consists of pro- and anti-apoptotic members that interact antagonistically to determine cell fate. Survival signaling through AKT and mTOR increases the

anti-apoptotic family members and decreases the pro-apoptotic members. (B) By inhibiting mTOR activity the balance is shifted where anti-apoptotic family members are reduced and pro-apoptotic members are increased. This reduces the capacity of anti-apoptotic proteins to sequester the pro-apoptotic proteins. Addition of ABT-199, a BCL-2 inhibitor, further shifts this balance to release the pro-apoptotic proteins and cause cancer killing.

Table 1: Published trials of mTOR-targeted therapies in ALL and NHL

Study	Phase	Drug	Drug Class	Disease	Outcomes
Witzig et al. [67]	II	Temsirolimus	Rapalog	Relapsed MCL	13/34 ORR[1]
Ansell et al. [69]	II	Temsirolimus	Rapalog	Relapsed MCL	11/27 ORR
Hess et al. [70]	III	Temsirolimus	Rapalog	Relapsed/refractory MCL	12/54 ORR compared to 2/54 ORR for investigator's choice
Tobinai et al. [173]	I	Everolimus	Rapalog	Relapsed/refractory NHL	4/13 ORR
Smith et al. [73]	II	Temsirolimus	Rapalog	DLBCL	9/32 ORR
				FL	21/39 ORR
Witzig et al. [72]	II	Everolimus	Rapalog	DLBCL	14/47 ORR
				MCL	6/19 ORR
				FL	3/8 ORR
Rizzieri et al. [68]	II	Ridaforolimus	Rapalog	ALL	0/1 ORR
				MCL	3/9 ORR
Rheingold et al. [56]	I	Sirolimus	Rapalog	Relapsed/refractory ALL	3/9 ORR
Rheingold et al. [57]	I	Temsirolimus with intensive re-induction chemotherapy	Rapalog	Pediatric relapsed ALL	7/15 CR with 3/7 MRD < 0.01%. High toxicities.
Daver et al. [58]	I/II	Everolimus with hyper-CVAD	Rapalog	Relapsed/refractory ALL	8/24 ORR
Ansell et al. [71]	II	Temsirolimus with rituximab	Rapalog	Relapsed/refractory MCL	41/69
Oki et al. [152]	I	Everolimus with panobinostat	Rapalog	Relapsed/refractory NHL	10/30 ORR
Barnes et al. [174]	II	Everolimus with rituximab	Rapalog	DLBCL	9/24 ORR
Infante et al. [107]	I	MLN0128	TOR-KI	Multiple	Dose escalation
Basu et al. [105]	I	AZD2014	TOR-KI	Multiple	Dose escalation
Bendell et al. [106]	I	CC-223	TOR-KI	Multiple	Dose escalation

[1] Overall response rate

Table 2: Ongoing trials of mTOR targeted therapies/combinations in ALL and NHL

mTOR-targeted drug	Combination	Study	Phase	Disease
Everolimus		NCT00790036	III	DLBCL
Everolimus	Panobinostat	NCT00918333	I/II	NHL
Everolimus	Panobinostat	NCT00978432	II	DLBCL
Everolimus	Multiagent re-induction	NCT01523977	I	ALL
Temsirolimus	Etoposide and cyclophosphamide	NCT01614197	I	Relapsed ALL and NHL
Sirolimus	Hyper-CVAD	NCT01184885	I	Relapsed/refractory ALL
Temsirolimus	Rituximab and DHAP	NCT01653067	II	Relapsed/refractory DLBCL
Everolimus	Bortezomib	NCT00671112	I	Relapsed/refractory lymphoma
Temsirolimus	Rituximab and bendamustine	NCT01078142	I/II	Relapsed FL and MCL
Temsirolimus	Bortezomib	NCT01281917	II	Relapsed/Refractory NHL
Temsirolimus	Rituximab and cladribine	NCT00787969	I/II	Newly diagnosed MCL
Sirolimus	Multiagent chemotherapy	NCT01658007	I	Relapsed/refractory ALL and lymphoma
Everolimus	Rituximab	NCT01665768	II	Lymphoma
Temsirolimus	Vinblastine	NCT02343718	I	Recurrent/refractory lymphoma
Temsirolimus	Inotuzumab Ozogamicin	NCT01535989	I	Relapsed/refractory NHL
CC-115		NCT01353625	I	NHL and solid tumors
MLN0128		NCT02484430	II	Relapsed/refractory ALL
PQR309		NCT02249429	I	Relapsed/refractory lymphoma
CC-223		NCT01177397	I/II	NHL and solid tumors
CC-223	Rituximab	NCT02031419	I	DLBCL

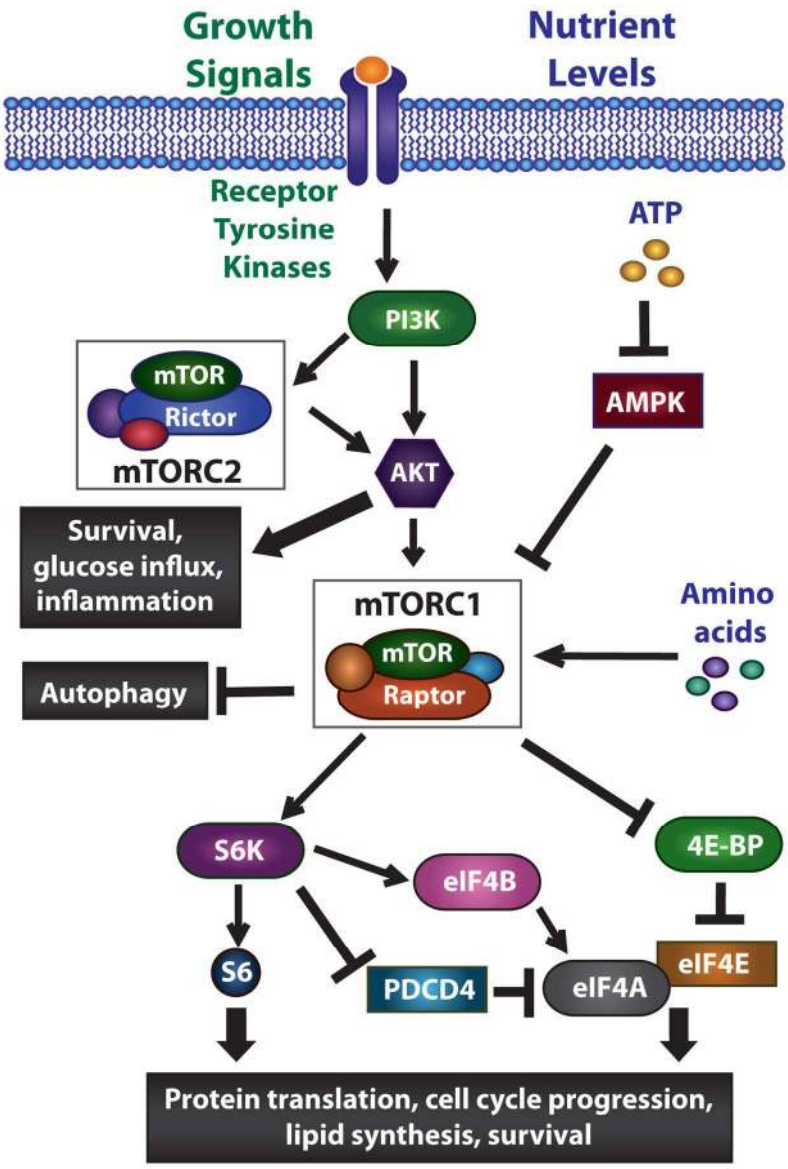

Figure 1
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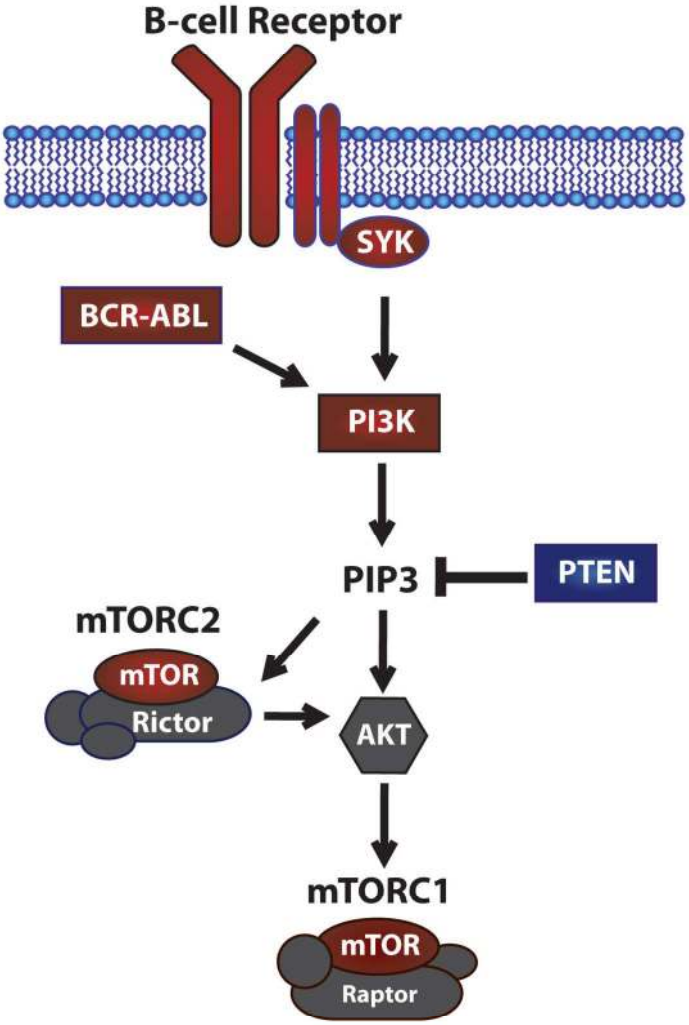

Figure 2
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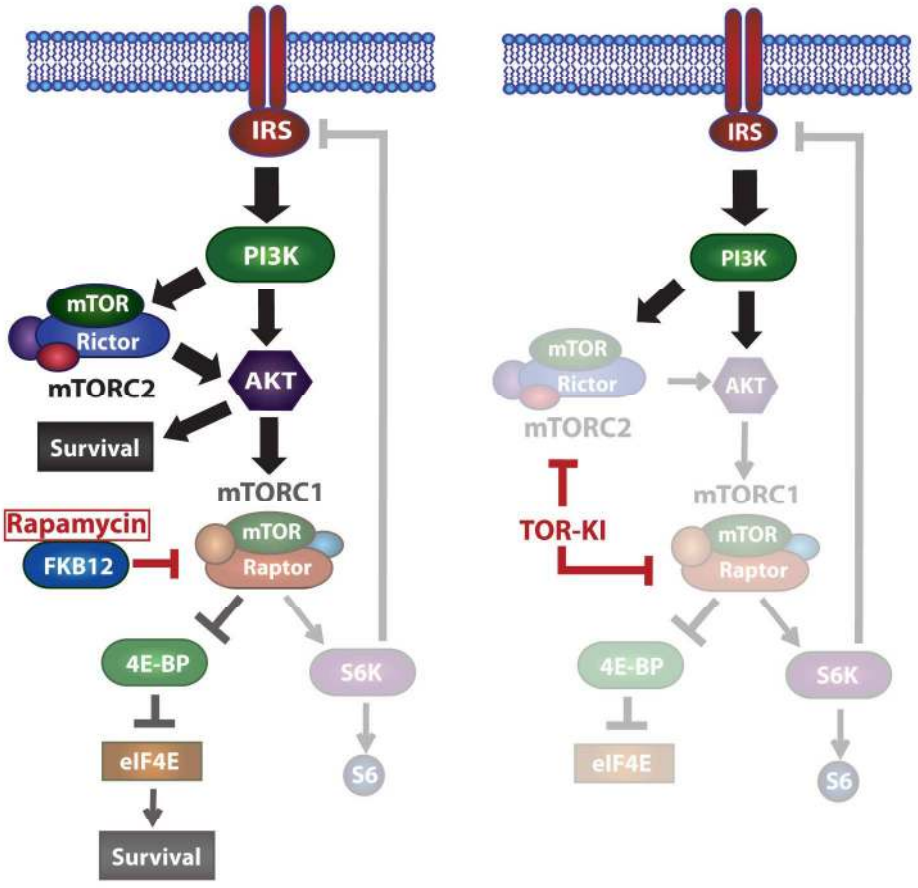

Figure 3
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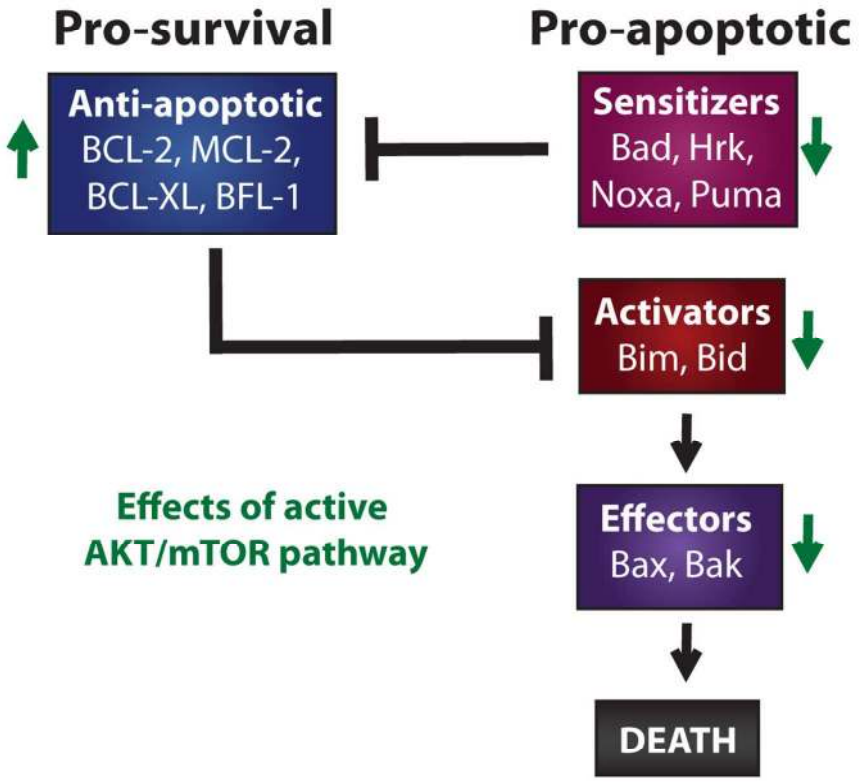

Figure 4A
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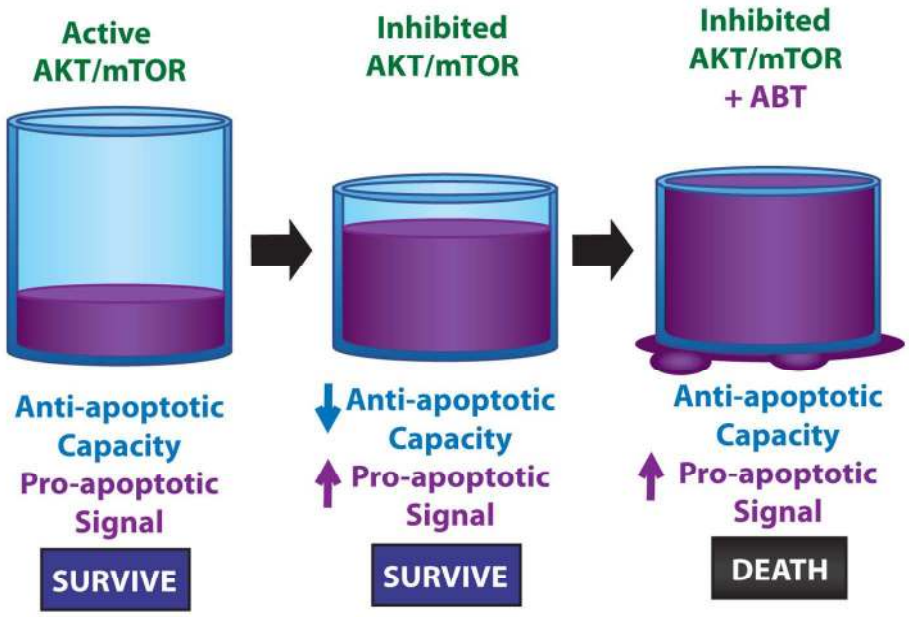

Figure 4B
132x96mm (300 x 300 DPI)