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Targeting mTOR for the treatment of B cell malignancies

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Abstract:	<p>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.</p>

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January 19, 2016

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,

A handwritten signature in black ink that reads '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 inhibition 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.*

1 **Targeting mTOR for the treatment of B cell malignancies**

2
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15
16 Running head: mTOR inhibitors in B cell malignancies

17
18 Keywords: mTOR, rapamycin, rapalogs, TOR-KIs, leukemia, lymphoma

19
20 Word count: 4,574

21 Figure count: 4

22 Table count: 2

23 Abstract

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

40 Introduction

41 *The mTOR Signaling Pathway*

42 mTOR is a serine/threonine kinase that functions as a master regulator of cell growth,
43 proliferation, metabolism, and survival. mTOR is active in two distinct multi-protein complexes
44 (mTORC1 and mTORC2) that are characterized by the defining subunits Raptor and
45 Rictor respectively [1,2]. Each complex is differentially regulated and has a distinct set of
46 substrates (Figure 1). Activation of mTORC2 is incompletely understood, but has recently been
47 shown to be dependent on the generation of PI(3,4,5)P₃ by phosphoinositide 3-kinase (PI3K)
48 [3]. Upon activation mTORC2 functions to amplify the activity of AKT, a key oncogene involved

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

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

67

68 *Evidence of mTOR activation in B-ALL and NHL*

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

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

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

91

92 **Rapamycin and Rapalogs: partial mTORC1 inhibitors**

93 *Mechanism of action*

94 Upon entry into a cell, rapamycin binds to FKBP12 forming a complex that potently and
95 selectively suppresses mTORC1 kinase activity by limiting substrate access to the active site
96 [39,40]. Importantly, the rapamycin-FKBP12 complex cannot bind to mTORC2 [2,41], though in
97 some cases prolonged exposure may limit the assembly of mTORC2 [42]. In this manner,
98 rapamycin behaves as a highly potent and selective inhibitor of mTORC1 (Figure 3). However,
99 poor solubility and pharmacokinetics spurred the development of rapamycin analogs (termed
100 rapalogs) for oral dosing in cancer patients [43]. Most notable among these rapalogs are

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

103

104 *Rapamycin and Rapalogs in B-ALL*

105 Early testing with rapamycin unveiled potent anti-proliferative efficacy in several preclinical
106 models of ALL. In an E μ -RET model of murine B-pre ALL, rapamycin as a single agent potently
107 inhibited proliferation of leukemia cells both *in vitro* and *in vivo* [47,48]. Similar efficacy was later
108 observed in models of Ph⁺ B-ALL [49,50] as well as in Ph-like B-ALL driven by JAK pathway
109 mutations or CRLF2 rearrangement [51]. Rapalogs also demonstrated marked preclinical
110 efficacy in primary human ALL samples grown *in vitro* or in xenograft models [50-52]. Notably,
111 rapamycin demonstrated single-agent cytotoxicity in primary pediatric ALL samples and
112 sensitized cells to doxorubicin *in vitro* [52]. Both everolimus and temsirolimus have shown
113 similar efficacy in xenograft models of adult and pediatric primary human ALL as single agents
114 [53] and in combination with chemotherapy [54,55].

115 Clinically, rapamycin as a single agent exhibited no dose-limiting toxicities, but had
116 lackluster efficacy compared to standard chemotherapeutic options (Table 1). In an early trial,
117 rapamycin yielded stable disease in only three out of nine pediatric patients with relapsed ALL
118 [56]. As a result, several trials have been launched to determine whether rapalogs can combine
119 safely and effectively with standard chemotherapies. An early pilot trial combining rapamycin
120 with glucocorticoids in relapsed ALL patients found that rapamycin effectively reduced the anti-
121 apoptotic protein MCL-1 in various patients. This promising outcome suggested that rapamycin
122 might sensitize ALL cells to apoptosis-inducing drugs. Indeed, in another study combining
123 temsirolimus with intensive multi-drug re-induction therapy (dexamethasone, mitoxantrone,
124 vincristine, and PEG-asparaginase) in relapsed childhood ALL yielded complete response in
125 seven of sixteen patients, of which three had less than 0.01% minimal residual disease (MRD)
126 by the end of treatment [57]. However, a separate trial evaluating everolimus combined with

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

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

144

145 *Rapalogs in NHL*

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

153 trial using a 10-fold lower dose of temsirolimus revealed that similar responses could be
154 obtained with lower toxicity [69]. Based on these results, a randomized phase III trial was
155 conducted. Strikingly, the ORR and progression free survival were significantly higher in
156 patients treated with temsirolimus compared to investigator's choice agent. These results
157 ultimately led to approval for temsirolimus as a single agent therapy for relapsed/refractory MCL
158 in Europe [70]. A subsequent phase II trial has also been completed combining temsirolimus
159 with rituximab in relapsed/refractory MCL. Despite demonstrating higher response rates than
160 single agent temsirolimus, the combination was also associated with higher toxicities including
161 thrombocytopenia and neutropenia in a significant fraction of patients [71]. Rapalog
162 monotherapy has also elicited responses in a subset of patients with other NHL subtypes. In a
163 phase II trial of everolimus in relapsed lymphoma, the ORR in DLBCL was 30% (14/47) and
164 38% (3/8) in FL [72]. Similar results were seen with temsirolimus where the ORR was 28% for
165 DLBCL and 53% in FL [73]. While these studies highlight that rapalogs have some activity, the
166 availability of better therapeutic options in both DLBCL and FL has limited the clinical progress
167 of rapalogs in these diseases. Thus, across NHL subtypes it will be important to determine
168 whether the addition of rapalogs to standard chemotherapy can provide additional benefit to
169 patients, without increasing toxicities.

170

171 *Outlook:*

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

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

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

202

203 **TOR-KIs: complete mTORC1/2 inhibitors**

204 The timely development of mTOR kinase inhibitors (TOR-KIs) directly addressed the
205 biochemical disadvantages of rapalogs. By competing with ATP for binding to the mTOR active
206 site, not only do TOR-KIs more completely block mTORC1 substrate phosphorylation (namely
207 4E-BPs), but they also inhibit mTORC2 activity [87,88]. This results in reduced phosphorylation
208 of AKT at Ser473 (Figure 3), dampening the feedback activation of PI3K/AKT that is known to
209 limit rapalog efficacy [89-91]. It is important to note that by competing with ATP, TOR-KIs are
210 capable of inhibiting several kinases at higher doses, including the structurally related protein,
211 PI3K. Conversely, several compounds that are often used pre-clinically as PI3K inhibitors
212 (wortmannin, LY294002) directly inhibit mTORC1 and mTORC2 at similar concentrations. Thus,
213 it is important to fully understand the pharmacologic properties of ATP-competitive mTOR and
214 PI3K inhibitors when interpreting their preclinical and clinical efficacy.

215 Several structurally distinct mTOR-selective inhibitors have been reported and tested in
216 models of B cell malignancies. Most notable among them are PP242 [88], Torin1 [87], Ku-
217 0063794 [92], AZD8055 [93], AZD2014 [93], MLN0128 (previously INK128 [94]), and CC-223
218 [95]. In preclinical testing, these TOR-KIs proved superior to rapalogs in terms of cytostatic and
219 cytotoxic potential. For example, in a mouse model of AKT-driven lymphangiogenesis, PP242
220 strongly suppressed both 4E-BP1 phosphorylation and tumor growth compared to rapamycin
221 [96]. These findings were also recapitulated *in vitro* using leukemia and DLBCL cell lines where
222 TOR-KIs had a greatly improved biochemical effect on downstream 4E-BP phosphorylation [97-
223 99].

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

230 fully suppress mTOR activity, our lab has found that both AZD8055 and MLN0128 maintain a
231 primarily cytostatic response profile (that is greater than rapalogs) [98,102-104]. Notably, low
232 doses of PP242 were sufficient to kill murine bone marrow cells immortalized by p190-BCR-ABL
233 [99], suggesting that fully transformed B-ALL cells with additional oncogenic lesions may
234 respond differently to mTOR inhibition. Thus, it remains unclear whether TOR-KIs will be
235 effective in B-ALL or NHL as a single agents at doses that are highly selective for mTOR kinase
236 activity.

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

250

251 **Emerging Combinations with mTOR Inhibitors:**

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

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

260

261 *Combinations targeting resistance mechanisms*262 Targeting parallel and downstream pathways

263 As with all targeted therapies, an understanding of how cells maintain survival in the
264 presence of mTOR inhibitors has been crucial to the identification of promising combinations.
265 Currently, there are several known acquired and *de novo* mechanisms of resistance to mTOR-
266 targeted therapies. For example, in addition to feedback activation of PI3K/AKT, mTORC1
267 inhibition may also activate the parallel MAPK/ERK pathway in B-ALL. In a similar fashion,
268 PI3K/AKT/mTOR inhibition can also induce up-regulation of receptor tyrosine kinases (RTKs)
269 leading to resistance in some solid tumors [108]. In agreement with these induced resistance
270 mechanisms, the addition of MAPK inhibitors and RTK inhibitors have demonstrated
271 significantly more efficacy in combination with both rapalogs and TOR-KIs in preclinical settings
272 [80,109,110]. However, in other instances resistance to mTOR inhibition may be a result of
273 sustained downstream effector activity, particularly cap-dependent translation. For example, our
274 laboratory has noted resistance to TOR-KIs in DLBCL cell lines lacking expression of 4E-BPs
275 [98] or over-expressing eIF4E [111]. Furthermore, recent evidence has indicated that PIM and
276 MNK kinases can maintain cap-dependent translation downstream of mTORC1 inhibition [112].
277 In these situations, targeting cap-dependent translation indirectly using combinations of PIM or
278 MNK inhibitors with TOR-KIs has shown cytotoxic activity in AML cell lines [113,114] as well as
279 in cutaneous T cell lymphoma cell lines *in vitro* [115]. Additional work is required to evaluate the
280 potential of directly targeting the cap-dependent translation initiation machinery. It is likely that

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

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

295

296 Targeting apoptosis

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

307 ABT-737, and its orally bioavailable analog, ABT-263, represent the most potent and
308 selective small molecule inhibitors of BCL-2 and BCL-X_L. Both of these compounds
309 demonstrated remarkable cytotoxic potential that was significantly enhanced when combined
310 with mTOR inhibitors in DLBCL [121], FL [122], AML [123], and B-ALL [124]. However, due to
311 on-target toxicity associated with BCL-X_L inhibition [125], a more promising clinical candidate is
312 ABT-199 [126]. ABT-199 is a selective inhibitor of BCL-2 and has elicited substantial clinical
313 responses in patients with CLL as a single agent [127], leading to its designation as a
314 breakthrough therapy for CLL patients with a 17p deletion (p53). Importantly, we and others
315 have recently reported that ABT-199 synergizes with mTOR inhibition comparably to dual BCL-
316 2/BCL-X_L inhibitors [104,128], suggesting that the rationale established using first generation
317 BCL-2 antagonists will hold true for ABT-199. However, a key concern is whether the addition of
318 TOR-KIs to BCL-2 antagonists will enhance its toxicity towards non-cancer cells. In an effort to
319 address this question, our lab has recently demonstrated that the combination does not
320 synergize to kill peripheral blood mononuclear cells obtained from normal healthy donors [104].
321 Further work must be done to ensure that these potent combinations will maintain favorable
322 tolerability when administered to patients.

323

324

325 *mTOR inhibition as an adjuvant*326 Targeting oncogenic drivers

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

333 primarily cytostatic [129,130]. In the activated B cell like (ABC) subtype of DLBCL, which is
334 driven by sustained activation of the B cell receptor (BCR) [34], inhibition of the downstream
335 kinase, Bruton's tyrosine kinase (BTK), also synergized strongly with PI3K/AKT/mTOR inhibitors
336 [131]. However, the limitations of this approach are also becoming apparent. In particular, the
337 germinal center B cell-like (GCB) DLBCL subtype is unresponsive to combinations of BTK and
338 mTOR inhibitors likely because BCR activation is not an oncogenic driver in this setting [132].
339 More alarmingly, in some cases the addition of mTOR inhibitors may antagonize the effects of
340 other agents either through suppression of proliferation or through induction of autophagy
341 [133,134]. Studies like these serve as powerful reminders that a sound biological understanding
342 supporting the use of these combinations must precede their clinical use.

343

344 Targeting histone deacetylases (HDACs)

345 HDAC inhibitors are another promising class of drug that may benefit from the addition of
346 mTOR inhibitors. In addition to modulating histone function and gene expression, HDACs also
347 regulate the activity of non-histone proteins with relevance to B cell cancers (e.g. STAT, Hsp90,
348 and FOXO) [135-138]. Importantly, mutations in genes regulating protein acetylation have been
349 described in both B-ALL and NHL. For example, mutations in the CREBBP histone
350 acetyltransferase (HAT) domain have been identified in a subset of patients with relapsed
351 pediatric B-ALL where it may confer glucocorticoid resistance [139]. Similar mutations in HAT
352 activity were identified as frequent mutations in both FL and DLBCL where their inactivation
353 promotes aberrant up-regulation of BCL-6, a protein known to promote B cell malignancies
354 [140-142]. Given the pervasive importance of protein acetylation, it is unsurprising that HDAC
355 inhibitors have elicited promising responses in various leukemias and lymphomas. For example,
356 in lymphomas with a t(14;18) translocation, HDAC inhibitors were shown to markedly reduce
357 expression of BCL-2 leading to apoptosis [143]. In other contexts, HDAC inhibition can induce
358 mitochondrial apoptosis via epigenetic regulation of other BCL-2 family proteins [144,145],

359 production of reactive oxygen species and ceramide [146], or activation of death receptors
360 [147]. Potent anti-proliferative effects have also been described [145,148]. Importantly, recent
361 evidence has suggested that the addition of mTOR inhibition may augment the effects of HDAC
362 inhibitors. For example, our lab has recently identified synergy between HDAC inhibitors and
363 TOR-KIs in B-ALL cell lines and primary patient samples [103]. Also, both temsirolimus and
364 everolimus have demonstrated synergistic anti-proliferative and apoptotic effects when
365 combined with the HDAC inhibitors in MCL [149,150]. In DLBCL, combining HDAC inhibitors
366 with rapalogs or TOR-KIs also synergistically induced apoptosis [65,151]. While there is still
367 debate as to the exact mechanism of synergy, it is clear that in a preclinical setting this
368 combination has marked potential in B cell malignancies. However, in a phase I trial combining
369 panobinostat and everolimus in relapsed/refractory lymphoma, the combination yielded ORRs
370 similar to everolimus alone but with higher incidence of thrombocytopenia [152]. As this
371 combination moves forward, it will be important to identify the exact mechanism of action so as
372 to better predict which patients may benefit from these combinations. It may also be useful to
373 explore compounds targeting selected subsets of cellular HDAC enzymes.

374

375 Targeting the proteasome

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

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

390

391 **Outlook**

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

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

412

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417

418

419 **Conflict of Interest Statement**

420 All authors have completed the Unified Competing Interest form at

421 http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author)

422 and declare: no support from any organisation for the submitted work; no financial relationships

423 with any organisations that might have an interest in the submitted work in the previous 3 years.

424 D.A.F. reports a patent, mTOR modulators and uses thereof, licensed to Intellikine, Inc.

425 **References**

- 426 1. Kim D-H, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P,
427 Sabatini DM. mTOR Interacts with Raptor to Form a Nutrient-Sensitive Complex that
428 Signals to the Cell Growth Machinery. *Cell*. 2002;110(2):163–75.
- 429 2. Sarbassov DD, Ali SM, Kim D-H, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst
430 P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-
431 insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*.
432 2004;14(14):1296–302.
- 433 3. Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, Wang B, Blenis J, Cantley LC, Toker
434 A, Su B, Wei W. PtdIns(3,4,5)P₃-Dependent Activation of the mTORC2 Kinase
435 Complex. *Cancer Discov*. 2015;5(11):1194–209.
- 436 4. Sarbassov DD. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR
437 Complex. *Science*. 2005;307(5712):1098–101.
- 438 5. Marte BM, Downward J. PKB/Akt: connecting phosphoinositide 3-kinase to cell survival
439 and beyond. *Trends Biochem Sci*. 1997;22(9):355–8.
- 440 6. Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD,
441 Chantranupong L, Comb W, Wang T, Bar-Peled L, Zoncu R, Straub C, Kim C, Park J,
442 Sabatini BL, Sabatini DM. Lysosomal amino acid transporter SLC38A9 signals arginine
443 sufficiency to mTORC1. *Science*. 2015;347(6218):188–94.
- 444 7. Rebsamen M, Pochini L, Stasyk T, de Araújo MEG, Galluccio M, Kandasamy RK,
445 Snijder B, Fauster A, Rudashevskaya EL, Bruckner M, Scorzoni S, Filipek PA, Huber
446 KVM, Bigenzahn JW, Heinz LX, Kraft C, Bennett KL, Indiveri C, Huber LA, Superti-
447 Furga G. SLC38A9 is a component of the lysosomal amino acid sensing machinery that
448 controls mTORC1. *Nature*. 2015;519(7544):477–81.
- 449 8. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer,
450 diabetes and ageing. *Nat Rev Mol Cell Biol*. 2010;12(1):21–35.
- 451 9. Laplante M, Sabatini DM. mTOR Signaling in Growth Control and Disease. *Cell*.
452 2012;149(2):274–93.
- 453 10. Hay N. Upstream and downstream of mTOR. *Genes Dev*. 2004;18(16):1926–45.
- 454 11. Kamada Y, Yoshino K-I, Kondo C, Kawamata T, Oshiro N, Yonezawa K, Ohsumi Y. Tor
455 directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol*.
456 2010;30(4):1049–58.
- 457 12. Chang Y-Y, Neufeld TP. An Atg1/Atg13 complex with multiple roles in TOR-mediated
458 autophagy regulation. *Mol Biol Cell*. 2009;20(7):2004–14.
- 459 13. Schmid T, Jansen AP, Baker AR, Hegamyer G, Hagan JP, Colburn NH. Translation
460 inhibitor Pdcd4 is targeted for degradation during tumor promotion. *Cancer Res*.
461 2008;68(5):1254–60.

- 462 14. Shahbazian D, Roux PP, Mieulet V, Cohen MS, Raught B, Taunton J, Hershey JWB,
463 Blenis J, Pende M, Sonenberg N. The mTOR/PI3K and MAPK pathways converge on
464 eIF4B to control its phosphorylation and activity. *EMBO J.* 2006;25(12):2781–91.
- 465 15. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF,
466 Aebersold R, Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step
467 mechanism. *Genes Dev.* 1999;13(11):1422–37.
- 468 16. Mamane Y, Petroulakis E, LeBacquer O, Sonenberg N. mTOR, translation initiation and
469 cancer. *Oncogene.* 2006;25(48):6416–22.
- 470 17. Bader AG, Kang S, Zhao L, Vogt PK. Oncogenic PI3K deregulates transcription and
471 translation. *Nat Rev Cancer.* 2005;5(12):921–9.
- 472 18. Grabiner BC, Nardi V, Birsoy K, Possemato R, Shen K, Sinha S, Jordan A, Beck AH,
473 Sabatini DM. A Diverse Array of Cancer-Associated MTOR Mutations Are
474 Hyperactivating and Can Predict Rapamycin Sensitivity. *Cancer Discov.* 2014;4(5):554–
475 63.
- 476 19. Pfeifer H, Wassmann B, Pavlova A, Wunderle L, Oldenburg J, Binckebanck A, Lange T,
477 Hochhaus A, Wystub S, Brück P, Hoelzer D, Ottmann OG. Kinase domain mutations of
478 BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients
479 with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood.*
480 2007;110(2):727–34.
- 481 20. Crist W, Carroll A, Shuster J, Jackson J, Head D, Borowitz M, Behm F, Link M, Steuber
482 P, Ragab A. Philadelphia chromosome positive childhood acute lymphoblastic
483 leukemia: clinical and cytogenetic characteristics and treatment outcome. A Pediatric
484 Oncology Group study. *Blood.* 1990;76(3):489–94.
- 485 21. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, Chen S-C, Payne-Turner D,
486 Churchman ML, Harvey RC, Chen X, Kasap C, Yan C, Becksfort J, Finney RP,
487 Teachey DT, Maude SL, Tse K, Moore R, Jones S, Mungall K, Birol I, Edmonson MN,
488 Hu Y, Buetow KE, Chen I-M, Carroll WL, Wei L, Ma J, Kleppe M, Levine RL, Garcia-
489 Manero G, Larsen E, Shah NP, Devidas M, Reaman G, Smith M, Paugh SW, Evans
490 WE, Grupp SA, Jeha S, Pui C-H, Gerhard DS, Downing JR, Willman CL, Loh M,
491 Hunger SP, Marra MA, Mullighan CG. Genetic Alterations Activating Kinase and
492 Cytokine Receptor Signaling in High-Risk Acute Lymphoblastic Leukemia. *Cancer Cell.*
493 2012;22(2):153–66.
- 494 22. Tasian SK, Doral MY, Borowitz MJ, Wood BL, Chen I-M, Harvey RC, Gastier-Foster
495 JM, Willman CL, Hunger SP, Mullighan CG, Loh ML. Aberrant STAT5 and PI3K/mTOR
496 pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic
497 leukemia. *Blood.* 2012;120(4):833–42.
- 498 23. Morishita N, Tsukahara H, Chayama K, Ishida T, Washio K, Miyamura T, Yamashita N,
499 Oda M, Morishima T. Activation of Akt is associated with poor prognosis and
500 chemotherapeutic resistance in pediatric B-precursor acute lymphoblastic leukemia.
501 *Pediatr Blood Cancer.* 2011;59(1):83–9.
- 502 24. Gomes AM, Soares MVD, Ribeiro P, Caldas J, Póvoa V, Martins LR, Melão A, Serra-

- 503 Caetano A, de Sousa AB, Lacerda JF, Barata JT. Adult B-cell acute lymphoblastic
504 leukemia cells display decreased PTEN activity and constitutive hyperactivation of
505 PI3K/Akt pathway despite high PTEN protein levels. *Haematologica*. 2014;99(6):1062–
506 8.
- 507 25. Nemes K, Sebestyén A, Márk Á, Hajdu M, Kenessey I, Sticz T, Nagy E, Barna G,
508 Váradi Z, Kovács G, Kopper L, Csóka M. Mammalian target of rapamycin (mTOR)
509 activity dependent phospho-protein expression in childhood acute lymphoblastic
510 leukemia (ALL). Bendall L, editor. *PLoS One*. 2013;8(4):e59335.
- 511 26. Peponi E, Drakos E, Reyes G, Leventaki V, Rassidakis GZ, Medeiros LJ. Activation of
512 mammalian target of rapamycin signaling promotes cell cycle progression and protects
513 cells from apoptosis in mantle cell lymphoma. *American J Pathol*. 2006;169(6):2171–
514 80.
- 515 27. Rudelius M, Pittaluga S, Nishizuka S, Pham THT, Fend F, Jaffe ES, Quintanilla-
516 Martinez L, Raffeld M. Constitutive activation of Akt contributes to the pathogenesis and
517 survival of mantle cell lymphoma. *Blood*. 2006;108(5):1668–76.
- 518 28. Uddin S, Hussain AR, Siraj AK, Manogaran PS, Al-Jomah NA, Moorji A, Atizado V, Al-
519 Dayel F, Belgaumi A, El-Solh H, Ezzat A, Bavi P, Al-Kuraya KS. Role of
520 phosphatidylinositol 3'-kinase/AKT pathway in diffuse large B-cell lymphoma survival.
521 *Blood*. 2006;108(13):4178–86.
- 522 29. Hasselblom S, Hansson U, Olsson M, Torén L, Bergström A, Nilsson-Ehle H,
523 Andersson P-O. High immunohistochemical expression of p-AKT predicts inferior
524 survival in patients with diffuse large B-cell lymphoma treated with
525 immunochemotherapy. *Br J Haematol*. 2010;149(4):560–8.
- 526 30. Yu B-H, Zhou X-Y, Xiao X-Y, Yan S-Y, Qin T, Shi D-R. [Activation and clinicopathologic
527 significance of AKT/mTOR signaling pathway in diffuse large B-cell lymphoma].
528 *Zhonghua Bing Li Xue Za Zhi*. 2009;38(1):35–41.
- 529 31. Psyrris A, Papageorgiou S, Liakata E, Scorilas A, Rontogianni D, Kontos CK, Argyriou P,
530 Pectasides D, Harhalakis N, Pappa V, Kolialexi A, Economopoulou C, Kontsioti F,
531 Maratou E, Dimitriadis G, Economopoulou P, Economopoulos T. Phosphatidylinositol
532 3'-kinase catalytic subunit alpha gene amplification contributes to the pathogenesis of
533 mantle cell lymphoma. *Clin Cancer Res*. 2009;15(18):5724–32.
- 534 32. Baohua Y, Xiaoyan Z, Tiecheng Z, Tao Q, Daren S. Mutations of the PIK3CA gene in
535 diffuse large B cell lymphoma. *Diagn Mol Pathol*. 2008;17(3):159–65.
- 536 33. Abubaker J, Bavi PP, Al-harbi S, Siraj AK, Al-Dayel F, Uddin S, Al-Kuraya K. PIK3CA
537 mutations are mutually exclusive with PTEN loss in diffuse large B-cell lymphoma.
538 *Leukemia*. 2007;21(11):2368–70.
- 539 34. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, Kohlhammer H, Lamy
540 L, Zhao H, Yang Y, Xu W, Shaffer AL, Wright G, Xiao W, Powell J, Jiang J-K, Thomas
541 CJ, Rosenwald A, Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Johnson
542 NA, Rimsza LM, Campo E, Jaffe ES, Wilson WH, Delabie J, Smeland EB, Fisher RI,
543 Brazier RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Pierce SK, Staudt LM.

- 544 Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*.
545 2010;463(7277):88–92.
- 546 35. Leseux L, Laurent G, Laurent C, Rigo M, Blanc A, Olive D, Bezombes C. PKC zeta
547 mTOR pathway: a new target for rituximab therapy in follicular lymphoma. *Blood*.
548 2008;111(1):285–91.
- 549 36. Fruchon S, Kheirallah S, Saati Al T, Ysebaert L, Laurent C, Leseux L, Fournié JJ,
550 Laurent G, Bezombes C. Involvement of the Syk-mTOR pathway in follicular lymphoma
551 cell invasion and angiogenesis. *Leukemia*. 2012;26(4):795–805.
- 552 37. Leseux L, Hamdi SM, Saati al T, Capilla F, Récher C, Laurent G, Bezombes C. Syk-
553 dependent mTOR activation in follicular lymphoma cells. *Blood*. 2006;108(13):4156–62.
- 554 38. Gulmann C, Espina V, Petricoin E, Longo DL, Santi M, Knutsen T, Raffeld M, Jaffe ES,
555 Liotta LA, Feldman AL. Proteomic analysis of apoptotic pathways reveals prognostic
556 factors in follicular lymphoma. *Clin Cancer Res*. 2005;11(16):5847–55.
- 557 39. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex
558 interacting with the binding domain of human FRAP. *Science*. 1996;273(5272):239–42.
- 559 40. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP. mTOR kinase
560 structure, mechanism and regulation. *Nature*. 2013;497(7448):217–23.
- 561 41. Jacinto E, Loewith R, Schmidt A, Lin S, Rüegg MA, Hall A, Hall MN. Mammalian TOR
562 complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*.
563 2004;6(11):1122–8.
- 564 42. Sarbassov DD, Ali SM, Sengupta S, Sheen J-H, Hsu PP, Bagley AF, Markhard AL,
565 Sabatini DM. Prolonged Rapamycin Treatment Inhibits mTORC2 Assembly and
566 Akt/PKB. *Mol Cell*. 2006;22(2):159–68.
- 567 43. Li J, Kim SG, Blenis J. Rapamycin: one drug, many effects. *Cell Metab*.
568 2014;19(3):373–9.
- 569 44. Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. *Clin Cancer Res*.
570 2008;14(5):1286–90.
- 571 45. Gabardi S, Baroletti SA. Everolimus: a proliferation signal inhibitor with clinical
572 applications in organ transplantation, oncology, and cardiology. *Pharmacotherapy*.
573 2010;30(10):1044–56.
- 574 46. Mita M, Sankhala K, Abdel-Karim I, Mita A, Giles F. Deforolimus (AP23573) a novel
575 mTOR inhibitor in clinical development. *Expert Opin Investig Drugs*. 2008;17(12):1947–
576 54.
- 577 47. Brown VI, Fang J, Alcorn K, Barr R, Kim JM, Wasserman R, Grupp SA. Rapamycin is
578 active against B-precursor leukemia in vitro and in vivo, an effect that is modulated by
579 IL-7-mediated signaling. *Proc Natl Acad Sci U S A*. 2003;100(25):15113–8.
- 580 48. Brown VI, Hulitt J, Fish J, Sheen C, Bruno M, Xu Q, Carroll M, Fang J, Teachey D,

- 581 Grupp SA. Thymic stromal-derived lymphopoietin induces proliferation of pre-B
582 leukemia and antagonizes mTOR inhibitors, suggesting a role for interleukin-7/alpha
583 signaling. *Cancer Res.* 2007;67(20):9963–70.
- 584 49. Kharas MG, Deane JA, Wong S, O'Bosky KR, Rosenberg N, Witte ON, Fruman DA.
585 Phosphoinositide 3-kinase signaling is essential for ABL oncogene-mediated
586 transformation of B-lineage cells. *Blood.* 2004;103(11):4268–75.
- 587 50. Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, Fruman DA.
588 Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR
589 inhibitor prevents expansion of human BCR-ABL+ leukemia cells. *J Clin Invest.*
590 2008;118(9):3038–50.
- 591 51. Maude SL, Tasian SK, Vincent T, Hall JW, Sheen C, Roberts KG, Seif AE, Barrett DM,
592 Chen I-M, Collins JR, Mullighan CG, Hunger SP, Harvey RC, Willman CL, Fridman JS,
593 Loh ML, Grupp SA, Teachey DT. Targeting JAK1/2 and mTOR in murine xenograft
594 models of Ph-like acute lymphoblastic leukemia. *Blood.* 2012;120(17):3510–8.
- 595 52. Avellino R, Romano S, Parasole R, Bisogni R, Lamberti A, Poggi V, Venuta S, Romano
596 MF. Rapamycin stimulates apoptosis of childhood acute lymphoblastic leukemia cells.
597 *Blood.* 2005;106(4):1400–6.
- 598 53. Teachey DT, Obzut DA, Cooperman J, Fang J, Carroll M, Choi JK, Houghton PJ, Brown
599 VI, Grupp SA. The mTOR inhibitor CCI-779 induces apoptosis and inhibits growth in
600 preclinical models of primary adult human ALL. *Blood.* 2006;107(3):1149–55.
- 601 54. Teachey DT, Sheen C, Hall J, Ryan T, Brown VI, Fish J, Reid GSD, Seif AE, Norris R,
602 Chang YJ, Carroll M, Grupp SA. mTOR inhibitors are synergistic with methotrexate: an
603 effective combination to treat acute lymphoblastic leukemia. *Blood.* 2008;112(5):2020–
604 3.
- 605 55. Crazzolaro R, Cisterne A, Thien M, Hewson J, Baraz R, Bradstock KF, Bendall LJ.
606 Potentiating effects of RAD001 (Everolimus) on vincristine therapy in childhood acute
607 lymphoblastic leukemia. *Blood.* 2009;113(14):3297–306.
- 608 56. Rheingold SR, Sacks N, Chang YJ, Brown VI, Teachey DT, Lange BJ, Grupp SA. A
609 Phase I Trial of Sirolimus (Rapamycin) in Pediatric Patients with Relapsed/Refractory
610 Leukemia. 2007;110(11):2834.
- 611 57. Rheingold SR, Whitlock JA, Tasian SK, Teachey DT, Borowitz MJ, Liu X, Ahern CH,
612 Minard C, Fox E, Weigel B, Blaney S. Temsirolimus and intensive re-induction
613 chemotherapy for 2nd or greater relapse of acute lymphoblastic leukemia (ALL): A
614 Children's Oncology Group study. 2015 ASCO Annual Meeting. Chicago, IL.
- 615 58. Daver N, Bumber Y, Kantarjian H, Ravandi F, Cortes J, Rytting ME, Kawedia JD,
616 Basnett J, Culotta KS, Zeng Z, Lu H, Richie MA, Garris R, Xiao L, Liu W, Baggerly KA,
617 Jabbour E, O'Brien S, Burger J, Bendall LJ, Thomas D, Konopleva M. A Phase I/II
618 Study of the mTOR Inhibitor Everolimus in Combination with HyperCVAD
619 Chemotherapy in Patients with Relapsed/Refractory Acute Lymphoblastic Leukemia.
620 *Clin Cancer Res.* 2015;21(12):2704–14.

- 621 59. O'Brien S, Thomas D, Ravandi F, Faderl S, Cortes J, Borthakur G, Pierce S, Garcia-
622 Manero G, Kantarjian HM. Outcome of adults with acute lymphocytic leukemia after
623 second salvage therapy. *Cancer*. 2008;113(11):3186–91.
- 624 60. Gökbüget N, Stanze D, Beck J, Diedrich H, Horst H-A, Hüttmann A, Kobbe G, Kreuzer
625 K-A, Leimer L, Reichle A, Schaich M, Schwartz S, Serve H, Starck M, Stelljes M,
626 Stuhlmann R, Viardot A, Wendelin K, Freund M, Hoelzer D, German Multicenter Study
627 Group for Adult Acute Lymphoblastic Leukemia. Outcome of relapsed adult
628 lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic
629 factors, and performance of stem cell transplantation. *Blood*. 2012;120(10):2032–41.
- 630 61. Place AE, Pikman Y, Stevenson K, Harris MH, Cooper TM, Gore L, Hijiya N, Loh ML,
631 Pauly M, Sulis M-L, Neuberg DS, Stegmaier K, Sallan SE, Silverman LB. Abstract 3765:
632 Phase Ib Trial of the mTOR Inhibitor Everolimus Given in Combination with Multiagent
633 Chemotherapy in Relapsed Acute Lymphoblastic Leukemia. 2015;126(23):3765.
- 634 62. Dal Col J, Zancai P, Terrin L, Guidoboni M, Ponzoni M, Pavan A, Spina M, Bergamin S,
635 Rizzo S, Tirelli U, De Rossi A, Doglioni C, Dolcetti R. Distinct functional significance of
636 Akt and mTOR constitutive activation in mantle cell lymphoma. *Blood*.
637 2008;111(10):5142–51.
- 638 63. Hipp S, Ringshausen I, Oelsner M, Bogner C, Peschel C, Decker T. Inhibition of the
639 mammalian target of rapamycin and the induction of cell cycle arrest in mantle cell
640 lymphoma cells. *Haematologica*. 2005;90(10):1433–4.
- 641 64. Gupta M, Dillon SR, Ziesmer SC, Feldman AL, Witzig TE, Ansell SM, Cerhan JR,
642 Novak AJ. A proliferation-inducing ligand mediates follicular lymphoma B-cell
643 proliferation and cyclin D1 expression through phosphatidylinositol 3-kinase-regulated
644 mammalian target of rapamycin activation. *Blood*. 2009;113(21):5206–16.
- 645 65. Gupta M, Ansell SM, Novak AJ, Kumar S, Kaufmann SH, Witzig TE. Inhibition of
646 histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B-cell
647 lymphoma by inhibiting Akt signaling through mTORC2. *Blood*. 2009;114(14):2926–35.
- 648 66. Wanner K, Hipp S, Oelsner M, Ringshausen I, Bogner C, Peschel C, Decker T.
649 Mammalian target of rapamycin inhibition induces cell cycle arrest in diffuse large B cell
650 lymphoma (DLBCL) cells and sensitises DLBCL cells to rituximab. *Br J Haematol*.
651 2006;134(5):475–84.
- 652 67. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, Ansell SM, Luyun R,
653 Flynn PJ, Morton RF, Dakhil SR, Gross H, Kaufmann SH. Phase II trial of single-agent
654 temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol*.
655 2005;23(23):5347–56.
- 656 68. Rizzieri DA, Feldman E, DiPersio JF, Gabrail N, Stock W, Strair R, Rivera VM, Albitar
657 M, Bedrosian CL, Giles FJ. A phase 2 clinical trial of deforolimus (AP23573, MK-8669),
658 a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory
659 hematologic malignancies. *Clin Cancer Res*. 2008;14(9):2756–62.
- 660 69. Ansell SM, Inwards DJ, Rowland KM, Flynn PJ, Morton RF, Moore DF, Kaufmann SH,
661 Ghobrial I, Kurtin PJ, Maurer M, Allmer C, Witzig TE. Low-dose, single-agent

- 662 temsirolimus for relapsed mantle cell lymphoma: a phase 2 trial in the North Central
663 Cancer Treatment Group. *Cancer*. 2008;113(3):508–14.
- 664 70. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, Laurell A,
665 Offner F, Strahs A, Berkenblit A, Hanushevsky O, Clancy J, Hewes B, Moore L, Coiffier
666 B. Phase III Study to Evaluate Temsirolimus Compared With Investigator's Choice
667 Therapy for the Treatment of Relapsed or Refractory Mantle Cell Lymphoma. *J Clin
668 Oncol*. 2009;27(23):3822–9.
- 669 71. Ansell SM, Tang H, Kurtin PJ, Koenig PA, Inwards DJ, Shah K, Ziesmer SC, Feldman
670 AL, Rao R, Gupta M, Erlichman C, Witzig TE. Temsirolimus and rituximab in patients
671 with relapsed or refractory mantle cell lymphoma: a phase 2 study. *Lancet Oncol*.
672 2011;12(4):361–8.
- 673 72. Witzig TE, Reeder CB, LaPlant BR, Gupta M, Johnston PB, Micallef IN, Porrata LF,
674 Ansell SM, Colgan JP, Jacobsen ED, Ghobrial IM, Habermann TM. A phase II trial of
675 the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. *Leukemia*.
676 2011;25(2):341–7.
- 677 73. Smith SM, van Besien K, Karrison T, Dancey J, McLaughlin P, Younes A, Smith S, Stiff
678 P, Lester E, Modi S, Doyle LA, Vokes EE, Pro B. Temsirolimus Has Activity in Non-
679 Mantle Cell Non-Hodgkin's Lymphoma Subtypes: The University of Chicago Phase II
680 Consortium. *J Clin Oncol*. 2010;28(31):4740–6.
- 681 74. Dilling MB, Dias P, Shapiro DN, Germain GS, Johnson RK, Houghton PJ. Rapamycin
682 selectively inhibits the growth of childhood rhabdomyosarcoma cells through inhibition
683 of signaling via the type I insulin-like growth factor receptor. *Cancer Res*.
684 1994;54(4):903–7.
- 685 75. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmithorst
686 VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN. Sirolimus for angiomyolipoma in
687 tuberous sclerosis complex or lymphangiomyomatosis. *N Engl J Med*.
688 2008;358(2):140–51.
- 689 76. Marsh DJ, Trahair TN, Martin JL, Chee WY, Walker J, Kirk EP, Baxter RC, Marshall
690 GM. Rapamycin treatment for a child with germline PTEN mutation. *Nat Clin Pract
691 Oncol*. 2008;5(6):357–61.
- 692 77. Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E,
693 Sauter M, Nonomura N, Brakemeier S, de Vries PJ, Whittmore VH, Chen D, Sahnoud
694 T, Shah G, Lincy J, Lebowhl D, Budde K. Everolimus for angiomyolipoma associated
695 with tuberous sclerosis complex or sporadic lymphangiomyomatosis (EXIST-2): a
696 multicentre, randomised, double-blind, placebo-controlled trial. *Lancet*.
697 2013;381(9869):817–24.
- 698 78. Santulli G, Totary-Jain H. Tailoring mTOR-based therapy: molecular evidence and
699 clinical challenges. *Pharmacogenomics*. 2013;14(12):1517–26.
- 700 79. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback
701 activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene*.
702 2007;26(13):1932–40.

- 703 80. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki
704 AT, THOMAS G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP.
705 Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent
706 feedback loop in human cancer. *J Clin Invest.* 2008;118(9):3065–74.
- 707 81. Sun S-Y, Rosenberg LM, Wang X, Zhou Z, Yue P, Fu H, Khuri FR. Activation of Akt and
708 eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin
709 inhibition. *Cancer Res.* 2005;65(16):7052–8.
- 710 82. O'Reilly KE, Rojo F, She Q-B, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin
711 DJ, Ludwig DL, Baselga J, Rosen N. mTOR inhibition induces upstream receptor
712 tyrosine kinase signaling and activates Akt. *Cancer Res.* 2006;66(3):1500–8.
- 713 83. Choo AY, Yoon S-O, Kim SG, Roux PP, Blenis J. Rapamycin differentially inhibits S6Ks
714 and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proc Natl
715 Acad Sci U S A.* 2008;105(45):17414–9.
- 716 84. Kang SA, Pacold ME, Cervantes CL, Lim D, Lou HJ, Ottina K, Gray NS, Turk BE, Yaffe
717 MB, Sabatini DM. mTORC1 phosphorylation sites encode their sensitivity to starvation
718 and rapamycin. *Science.* 2013;341(6144):1236566–6.
- 719 85. Ruggero D, Montanaro L, Ma L, Xu W, Londei P, Cordon-Cardo C, Pandolfi PP. The
720 translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in
721 lymphomagenesis. *Nat Med.* 2004;10(5):484–6.
- 722 86. Dowling RJO, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X,
723 Larsson O, Selvaraj A, Liu Y, Kozma SC, THOMAS G, Sonenberg N. mTORC1-
724 mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science.*
725 2010;328(5982):1172–6.
- 726 87. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM. Active-
727 Site Inhibitors of mTOR Target Rapamycin-Resistant Outputs of mTORC1 and
728 mTORC2. *PLoS Biol.* 2009;7(2):e1000038.
- 729 88. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T,
730 Sabatini DM, Gray NS. An ATP-competitive Mammalian Target of Rapamycin Inhibitor
731 Reveals Rapamycin-resistant Functions of mTORC1. *J Biol Chem.* 2009;284(12):8023–
732 32.
- 733 89. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B.
734 SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation
735 and substrate specificity. *Cell.* 2006;127(1):125–37.
- 736 90. Neri LM, Cani A, Martelli AM, Simioni C, Junghanss C, Tabellini G, Ricci F, Tazzari PL,
737 Pagliaro P, McCubrey JA, Capitani S. Targeting the PI3K/Akt/mTOR signaling pathway
738 in B-precursor acute lymphoblastic leukemia and its therapeutic potential. *Leukemia.*
739 2014;28(4):739–48.
- 740 91. Petrich AM, Leshchenko V, Kuo PY, Xia B, Thirukonda VK, Ulahannan N, Gordon S,
741 Fazzari MJ, Ye BH, Sparano JA, Parekh S. Akt Inhibitors MK-2206 and Nelfinavir
742 Overcome mTOR Inhibitor Resistance in Diffuse Large B-cell Lymphoma. *Clin Cancer*

- 743 Res. 2012;18(9):2534–44.
- 744 92. García-Martínez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, Alessi
745 DR. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR).
746 Biochem J. 2009;421(1):29–42.
- 747 93. Pike KG, Malagu K, Hummersone MG, Menear KA, Duggan HME, Gomez S, Martin
748 NMB, Ruston L, Pass SL, Pass M. Optimization of potent and selective dual mTORC1
749 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. Bioorg Med Chem
750 Lett. 2013;23(5):1212–6.
- 751 94. Jessen K, Wang S, Kessler L, Guo X, Kucharski J, Staunton J, Lan L, Elia M, Stewart J,
752 Brown J, Li L, Chan K, Martin M, Ren P, Rommel C, Liu Y. Abstract B148: INK128 is a
753 potent and selective TORC1/2 inhibitor with broad oral antitumor activity. Mol Cancer
754 Ther. 2009;8(Supplement 1):B148–8.
- 755 95. Mortensen DS, Fultz KE, Xu S, Xu W, Packard G, Khambatta G, Gamez JC, Leisten J,
756 Zhao J, Apuy J, Ghoreishi K, Hickman M, Narla RK, Bissonette R, Richardson S, Peng
757 SX, Perrin-Ninkovic S, Tran T, Shi T, Yang WQ, Tong Z, Cathers BE, Moghaddam MF,
758 Canan SS, Worland P, Sankar S, Raymon HK. CC-223, a Potent and Selective Inhibitor
759 of mTOR Kinase: In Vitro and In Vivo Characterization. Mol Cancer Ther.
760 2015;14(6):1295–305.
- 761 96. Hsieh AC, Costa M, Zollo O, Davis C, Feldman ME, Testa JR, Meyuhos O, Shokat KM,
762 Ruggero D. Genetic Dissection of the Oncogenic mTOR Pathway Reveals Druggable
763 Addiction to Translational Control via 4EBP-eIF4E. Cancer Cell. 2010;17(3):249–61.
- 764 97. Ono A, Oike R, Okuhashi Y, Takahashi Y, Itoh M, Nara N, Tohda S. Comparative
765 effects of PP242 and rapamycin on mTOR signalling and NOTCH signalling in leukemia
766 cells. Anticancer Res. 2013;33(3):809–13.
- 767 98. Mallya S, Fitch BA, Lee JS, So L, Janes MR, Fruman DA. Resistance to mTOR kinase
768 inhibitors in lymphoma cells lacking 4EBP1. Sobol RW, editor. PLoS ONE.
769 2014;9(2):e88865.
- 770 99. Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, Vu C, Lilly MB, Mallya S, Ong
771 ST, Konopleva M, Martin MB, Ren P, Liu Y, Rommel C, Fruman DA. Effective and
772 selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. Nat Med.
773 2010;16(2):205–13.
- 774 100. Gupta M, Hendrickson AEW, Yun SS, Han JJ, Schneider PA, Koh BD, Stenson MJ,
775 Wellik LE, Shing JC, Peterson KL, Flatten KS, Hess AD, Smith BD, Karp JE, Barr S,
776 Witzig TE, Kaufmann SH. Dual mTORC1/mTORC2 inhibition diminishes Akt activation
777 and induces Puma-dependent apoptosis in lymphoid malignancies. Blood.
778 2012;119(2):476–87.
- 779 101. Simioni C, Cani A, Martelli AM, Zauli G, Tabellini G, McCubrey J, Capitani S, Neri LM.
780 Activity of the novel mTOR inhibitor Torin-2 in B-precursor acute lymphoblastic
781 leukemia and its therapeutic potential to prevent Akt reactivation. Oncotarget.
782 2014;5(20):10034–47.

- 783 102. Janes MR, Vu C, Mallya S, Shieh MP, Limon JJ, Li L-S, Jessen KA, Martin MB, Ren P,
784 Lilly MB, Sender LS, Liu Y, Rommel C, Fruman DA. Efficacy of the investigational
785 mTOR kinase inhibitor MLN0128/INK128 in models of B-cell acute lymphoblastic
786 leukemia. *Leukemia*. 2013;27(3):586–94.
- 787 103. Beagle BR, Nguyen DM, Mallya S, Tang SS, Lu M, Zeng Z, Konopleva M, Vo T-T,
788 Fruman DA. mTOR kinase inhibitors synergize with histone deacetylase inhibitors to kill
789 B-cell acute lymphoblastic leukemia cells. *Oncotarget*. 2015;6(4):2088–100.
- 790 104. Lee JS, Tang SS, Ortiz V, Vo T-T, Fruman DA. MCL-1-independent mechanisms of
791 synergy between dual PI3K/mTOR and BCL-2 inhibition in diffuse large B cell
792 lymphoma. *Oncotarget*. 2015;6(34):35202-17.
- 793 105. Basu B, Dean E, Puglisi M, Greystoke A, Ong M, Burke W, Cavallin M, Bigley G,
794 Womack C, Harrington EA, Green S, Oelmann E, de Bono JS, Ranson M, Banerji U.
795 First-in-Human Pharmacokinetic and Pharmacodynamic Study of the Dual m-TORC 1/2
796 Inhibitor AZD2014. *Clin Cancer Res*. 2015;21(15):3412–9.
- 797 106. Bendell JC, Kelley RK, Shih KC, Grabowsky JA, Bergsland E, Jones S, Martin T,
798 Infante JR, Mischel PS, Matsutani T, Xu S, Wong L, Liu Y, Wu X, Mortensen DS,
799 Chopra R, Hege K, Munster PN. A phase I dose-escalation study to assess safety,
800 tolerability, pharmacokinetics, and preliminary efficacy of the dual mTORC1/mTORC2
801 kinase inhibitor CC-223 in patients with advanced solid tumors or multiple myeloma.
802 *Cancer*. 2015;121(19):3481–90.
- 803 107. Infante JR, Tabernero J, Cervantes A, Jalal S. Abstract C252: A phase 1, dose-
804 escalation study of MLN0128, an investigational oral mammalian target of rapamycin
805 complex 1/2 (mTORC1/2) catalytic inhibitor, in patients (pts) with advanced non-
806 hematologic malignancies. *Mol Cancer Ther*. 2013;12:C252.
- 807 108. Bertacchini J, Guida M, Accordi B, Mediani L, Martelli AM, Barozzi P, Petricoin E, Liotta
808 L, Milani G, Giordan M, Luppi M, Forghieri F, De Pol A, Cocco L, Basso G, Marmiroli S.
809 Feedbacks and adaptive capabilities of the PI3K/Akt/mTOR axis in acute myeloid
810 leukemia revealed by pathway selective inhibition and phosphoproteome analysis.
811 *Leukemia*. 2014;28(11):2197–205.
- 812 109. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M,
813 Moskatel E, Baselga J, Guichard S, Rosen N. mTOR kinase inhibition causes feedback-
814 dependent biphasic regulation of AKT signaling. *Cancer Discov*. 2011;1(3):248–59.
- 815 110. García-García C, Ibrahim YH, Serra V, Calvo MT, Guzmán M, Grueso J, Aura C, Pérez
816 J, Jessen K, Liu Y, Rommel C, Tabernero J, Baselga J, Scaltriti M. Dual mTORC1/2
817 and HER2 blockade results in antitumor activity in preclinical models of breast cancer
818 resistant to anti-HER2 therapy. *Clin Cancer Res*. 2012;18(9):2603–12.
- 819 111. Alain T, Sonenberg N, Topisirovic I. mTOR inhibitor efficacy is determined by the
820 eIF4E/4E-BP ratio. *Oncotarget*. 2012;3(12):1491–2.
- 821 112. Schatz JH, Oricchio E, Wolfe AL, Jiang M, Linkov I, Maragulia J, Shi W, Zhang Z,
822 Rajasekhar VK, Pagano NC, Porco JA, Teruya-Feldstein J, Rosen N, Zelenetz AD,
823 Pelletier J, Wendel H-G. Targeting cap-dependent translation blocks converging

- 824 survival signals by AKT and PIM kinases in lymphoma. *J Exp Med*. 2011;208(9):1799–
825 807.
- 826 113. Harada M, Benito J, Yamamoto S, Kaur S, Arslan D, Ramirez S, Jacamo R, Plataniias
827 L, Matsushita H, Fujimura T, Kazuno S, Kojima K, Tabe Y, Konopleva M. The novel
828 combination of dual mTOR inhibitor AZD2014 and pan-PIM inhibitor AZD1208 inhibits
829 growth in acute myeloid leukemia via HSF pathway suppression. *Oncotarget*.
830 2015;6(35):37930–47.
- 831 114. Aparicio CB, Renner O, Gomez-Casero E. Abstract A275: Co-targeting PIM and
832 PI3K/mTOR pathways with a single molecule: Novel orally available combined
833 PIM/PI3K and PIM/PI3K/mTOR kinase inhibitors. *Mol Cancer Ther*. 2013;12(11
834 Suppl):A275.
- 835 115. Marzec M, Liu X, Wysocka M, Rook AH, Odum N, Wasik MA. Simultaneous inhibition of
836 mTOR-containing complex 1 (mTORC1) and MNK induces apoptosis of cutaneous T-
837 cell lymphoma (CTCL) cells. *PLoS ONE*. 2011;6(9):e24849.
- 838 116. André F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S,
839 Arena F, Isaacs C, Yap Y-S, Papai Z, Lang I, Armstrong A, Lerzo G, White M, Shen K,
840 Litton J, Chen D, Zhang Y, Ali S, Taran T, Gianni L. Everolimus for women with
841 trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a
842 randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol*.
843 2014;15(6):580–91.
- 844 117. Shimizu T, Tolcher AW, Papadopoulos KP, Beeram M, Rasco DW, Smith LS, Gunn S,
845 Smetzer L, Mays TA, Kaiser B, Wick MJ, Alvarez C, Cavazos A, Mangold GL, Patnaik
846 A. The clinical effect of the dual-targeting strategy involving PI3K/AKT/mTOR and
847 RAS/MEK/ERK pathways in patients with advanced cancer. *Clin Cancer Res*.
848 2012;18(8):2316–25.
- 849 118. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis
850 J, Greenberg ME. Akt Promotes Cell Survival by Phosphorylating and Inhibiting a
851 Forkhead Transcription Factor. *Cell*. 1999;96(6):857–68.
- 852 119. Dijkers PF, Medema RH, Lammers J-WJ, Koenderman L, Coffey PJ. Expression of the
853 pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor
854 FKHR-L1. *Curr Biol*. 2000;10(19):1201–4.
- 855 120. Sarosiek KA, Chi X, Bachman JA, Sims JJ, Montero J, Patel L, Flanagan A, Andrews
856 DW, Sorger P, Letai A. BID Preferentially Activates BAK while BIM Preferentially
857 Activates BAX, Affecting Chemotherapy Response. *Molecular Cell*. 2013;51(6):751–65.
- 858 121. Coloff JL, Macintyre AN, Nichols AG, Liu T, Gallo CA, Plas DR, Rathmell JC. Akt-
859 Dependent Glucose Metabolism Promotes Mcl-1 Synthesis to Maintain Cell Survival
860 and Resistance to Bcl-2 Inhibition. *Cancer Res*. 2011;71(15):5204–13.
- 861 122. Ackler S, Xiao Y, Mitten MJ, Foster K, Oleksijew A, Refici M, Schlessinger S, Wang B,
862 Chemburkar SR, Bauch J, Tse C, Frost DJ, Fesik SW, Rosenberg SH, Elmore SW,
863 Shoemaker AR. ABT-263 and rapamycin act cooperatively to kill lymphoma cells in vitro
864 and in vivo. *Mol Cancer Ther*. 2008;7(10):3265–74.

- 865 123. Rahmani M, Aust MM, Attkisson E, Williams DC, Ferreira-Gonzalez A, Grant S. Dual
866 inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in
867 human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism.
868 *Cancer Res.* 2013;73(4):1340–51.
- 869 124. Iacovelli S, Ricciardi MR, Allegretti M, Mirabilli S, Licchetta R, Bergamo P, Rinaldo C,
870 Zeuner A, Foà R, Milella M, McCubrey JA, Martelli AM, Tafuri A. Co-targeting of Bcl-2
871 and mTOR pathway triggers synergistic apoptosis in BH3 mimetics resistant acute
872 lymphoblastic leukemia. *Oncotarget.* 2015;6(31):32089–103.
- 873 125. Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, Chu
874 Q, Giaccone G, Khaira D, Ramalingam SS, Ranson MR, Dive C, McKeegan EM, Chyla
875 BJ, Dowell BL, Chakravarty A, Nolan CE, Rudersdorf N, Busman TA, Mabry MH,
876 Krivoshik AP, Humerickhouse RA, Shapiro GI, Gandhi L. Phase II Study of Single-
877 Agent Navitoclax (ABT-263) and Biomarker Correlates in Patients with Relapsed Small
878 Cell Lung Cancer. *Clin Cancer Res.* 2012;18(11):3163–9.
- 879 126. Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding
880 H, Enschede SH, Fairbrother WJ, Huang DCS, Hymowitz SG, Jin S, Khaw SL, Kovar
881 PJ, Lam LT, Lee J, Maecker HL, Marsh KC, Mason KD, Mitten MJ, Nimmer PM,
882 Oleksijew A, Park CH, Park C-M, Phillips DC, Roberts AW, Sampath D, Seymour JF,
883 Smith ML, Sullivan GM, Tahir SK, Tse C, Wendt MD, Xiao Y, Xue JC, Zhang H,
884 Humerickhouse RA, Rosenberg SH, Elmore SW. ABT-199, a potent and selective BCL-
885 2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med.*
886 2013;19(2):202–8.
- 887 127. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, Kipps TJ,
888 Anderson M-A, Brown JR, Gressick L, Wong S, Dunbar M, Zhu M, Desai MB, Cerri E,
889 Enschede SH, Humerickhouse RA, Wierda WG, Seymour JF. Targeting BCL2 with
890 Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med.*
891 2015;:151206090218007.
- 892 128. Choudhary GS, Al-harbi S, Mazumder S, Hill BT, Smith MR, Bodo J, Hsi ED, Almasan
893 A. MCL-1 and BCL-xL-dependent resistance to the BCL-2 inhibitor ABT-199 can be
894 overcome by preventing PI3K/AKT/mTOR activation in lymphoid malignancies. *Cell*
895 *Death Dis.* 2015;6(1):e1593.
- 896 129. Fiskus W, Verstovsek S, Manshouri T, Smith JE, Peth K, Abhyankar S, McGuirk J,
897 Bhalla KN. Dual PI3K/AKT/mTOR inhibitor BEZ235 synergistically enhances the activity
898 of JAK2 inhibitor against cultured and primary human myeloproliferative neoplasm cells.
899 *Mol Cancer Ther.* 2013;12(5):577–88.
- 900 130. Bogani C, Bartalucci N, Martinelli S, Tozzi L, Guglielmelli P, Bosi A, Vannucchi AM,
901 Associazione Italiana per la Ricerca sul Cancro AGIMM Gruppo Italiano Malattie
902 Mieloproliferative. mTOR inhibitors alone and in combination with JAK2 inhibitors
903 effectively inhibit cells of myeloproliferative neoplasms. Tse W, editor. *PLoS ONE.*
904 2013;8(1):e54826.
- 905 131. Mathews Griner LA, Guha R, Shinn P, Young RM, Keller JM, Liu D, Goldlust IS, Yasgar
906 A, McKnight C, Boxer MB, Dubeau DY, Jiang J-K, Michael S, Mierzwa T, Huang W,
907 Walsh MJ, Mott BT, Patel P, Leister W, Maloney DJ, Leclair CA, Rai G, Jadhav A,

- 908 Peyser BD, Austin CP, Martin SE, Simeonov A, Ferrer M, Staudt LM, Thomas CJ. High-
909 throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill
910 activated B-cell-like diffuse large B-cell lymphoma cells. *Proc Natl Acad Sci U S A*.
911 2014;111(6):2349–54.
- 912 132. Ezell SA, Mayo M, Bihani T, Tepsuporn S, Wang S, Passino M, Grosskurth SE, Collins
913 M, Parmentier J, Reimer C, Byth KF. Synergistic induction of apoptosis by combination
914 of BTK and dual mTORC1/2 inhibitors in diffuse large B cell lymphoma. *Oncotarget*.
915 2014;5(13):4990–5001.
- 916 133. Huang S, Yang ZJ, Yu C, Sinicrope FA. Inhibition of mTOR kinase by AZD8055 can
917 antagonize chemotherapy-induced cell death through autophagy induction and down-
918 regulation of p62/sequestosome 1. *J Biol Chem*. 2011;286(46):40002–12.
- 919 134. White E, DiPaola RS. The double-edged sword of autophagy modulation in cancer. *Clin
920 Cancer Res*. 2009;15(17):5308–16.
- 921 135. Zhuang S. Regulation of STAT signaling by acetylation. *Cell Signal*. 2013;25(9):1924–
922 31.
- 923 136. Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, Rocha K, Kumaraswamy S,
924 Boyapalle S, Atadja P, Seto E, Bhalla K. Inhibition of histone deacetylase 6 acetylates
925 and disrupts the chaperone function of heat shock protein 90: a novel basis for
926 antileukemia activity of histone deacetylase inhibitors. *J Biol Chem*.
927 2005;280(29):26729–34.
- 928 137. Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimochi K, Fukamizu A. Acetylation of
929 Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. *Proc Natl Acad
930 Sci U S A*. 2005;102(32):11278–83.
- 931 138. van der Heide LP, Smidt MP. Regulation of FoxO activity by CBP/p300-mediated
932 acetylation. *Trends Biochem Sci*. 2005;30(2):81–6.
- 933 139. Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL,
934 Holmfeldt L, Collins-Underwood JR, Ma J, Buetow KH, Pui C-H, Baker SD, Brindle PK,
935 Downing JR. CREBBP mutations in relapsed acute lymphoblastic leukaemia. *Nature*.
936 2011; 471(7337):235–9.
- 937 140. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper
938 LH, Lerach S, Tang H, Ma J, Rossi D, Chadburn A, Murty VV, Mullighan CG, Gaidano
939 G, Rabadan R, Brindle PK, Dalla-Favera R. Inactivating mutations of acetyltransferase
940 genes in B-cell lymphoma. *Nature*. 2011;471(7337):189–95.
- 941 141. Bereshchenko OR, Gu W, Dalla-Favera R. Acetylation inactivates the transcriptional
942 repressor BCL6. *Nat Genet*. 2002;32(4):606–13.
- 943 142. Basso K, Dalla-Favera R. Germinal centres and B cell lymphomagenesis. *Nat Rev
944 Immunol*. 2015;15(3):172–84.
- 945 143. Duan H, Heckman CA, Boxer LM. Histone deacetylase inhibitors down-regulate bcl-2
946 expression and induce apoptosis in t(14;18) lymphomas. *Mol Cell Biol*.

- 947 2005;25(5):1608–19.
- 948 144. Zhao Y, Tan J, Zhuang L, Jiang X, Liu ET, Yu Q. Inhibitors of histone deacetylases
949 target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic
950 protein Bim. *Proc Natl Acad Sci U S A*. 2005;102(44):16090–5.
- 951 145. Sakajiri S, Kumagai T, Kawamata N, Saitoh T, Said JW, Koeffler HP. Histone
952 deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell
953 lines. *Exp Hematol*. 2005;33(1):53–61.
- 954 146. Rahmani M, Reese E, Dai Y, Bauer C, Payne SG, Dent P, Spiegel S, Grant S.
955 Coadministration of histone deacetylase inhibitors and perifosine synergistically induces
956 apoptosis in human leukemia cells through Akt and ERK1/2 inactivation and the
957 generation of ceramide and reactive oxygen species. *Cancer Res*. 2005;65(6):2422–32.
- 958 147. Insinga A, Monestiroli S, Ronzoni S, Gelmetti V, Marchesi F, Viale A, Altucci L, Nervi C,
959 Minucci S, Pelicci PG. Inhibitors of histone deacetylases induce tumor-selective
960 apoptosis through activation of the death receptor pathway. *Nat Med*. 2005;11(1):71–6.
- 961 148. Heider U, Kaiser M, Sterz J, Zavrski I, Jakob C, Fleissner C, Eucker J, Possinger K,
962 Sezer O. Histone deacetylase inhibitors reduce VEGF production and induce growth
963 suppression and apoptosis in human mantle cell lymphoma. *Eur J Haematol*.
964 2006;76(1):42–50.
- 965 149. Yazbeck VY, Buglio D, Georgakis GV, Li Y, Iwado E, Romaguera JE, Kondo S, Younes
966 A. Temsirolimus downregulates p21 without altering cyclin D1 expression and induces
967 autophagy and synergizes with vorinostat in mantle cell lymphoma. *Exp Hematol*.
968 2008;36(4):443–50.
- 969 150. Haritunians T, Mori A, O'Kelly J, Luong QT, Giles FJ, Koeffler HP. Antiproliferative
970 activity of RAD001 (everolimus) as a single agent and combined with other agents in
971 mantle cell lymphoma. *Leukemia*. 2007;21(2):333–9.
- 972 151. Rahmani M, Aust MM, Benson EC, Wallace L, Friedberg J, Grant S. PI3K/mTOR
973 inhibition markedly potentiates HDAC inhibitor activity in NHL cells through BIM- and
974 MCL-1-dependent mechanisms in vitro and in vivo. *Clin Cancer Res*.
975 2014;20(18):4849–60.
- 976 152. Oki Y, Buglio D, Fanale M, Fayad L, Copeland A, Romaguera J, Kwak LW, Pro B, de
977 Castro Faria S, Neelapu S, Fowler N, Hagemester F, Zhang J, Zhou S, Feng L, Younes
978 A. Phase I study of panobinostat plus everolimus in patients with relapsed or refractory
979 lymphoma. *Clin Cancer Res*. 2013;19(24):6882–90.
- 980 153. Almond JB, Cohen GM. The proteasome: a novel target for cancer chemotherapy.
981 *Leukemia*. 2002;16(4):433–43.
- 982 154. Pérez-Galán P, Roué G, Villamor N, Montserrat E, Campo E, Colomer D. The
983 proteasome inhibitor bortezomib induces apoptosis in mantle-cell lymphoma through
984 generation of ROS and Noxa activation independent of p53 status. *Blood*.
985 2006;107(1):257–64.

- 986 155. Strauss SJ, Higginbottom K, Jülicher S, Maharaj L, Allen P, Schenkein D, Lister TA, Joel
987 SP. The proteasome inhibitor bortezomib acts independently of p53 and induces cell
988 death via apoptosis and mitotic catastrophe in B-cell lymphoma cell lines. *Cancer Res.*
989 2007;67(6):2783–90.
- 990 156. O'Connor OA, Wright J, Moskowitz C, Muzzy J, MacGregor-Cortelli B, Stubblefield M,
991 Straus D, Portlock C, Hamlin P, Choi E, Dumetrescu O, Esseltine D, Trehu E, Adams J,
992 Schenkein D, Zelenetz AD. Phase II clinical experience with the novel proteasome
993 inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell
994 lymphoma. *J Clin Oncol.* 2005;23(4):676–84.
- 995 157. Goy A, Younes A, McLaughlin P, Pro B, Romaguera JE, Hagemester F, Fayad L, Dang
996 NH, Samaniego F, Wang M, Broglio K, Samuels B, Gilles F, Sarris AH, Hart S, Trehu E,
997 Schenkein D, Cabanillas F, Rodriguez AM. Phase II study of proteasome inhibitor
998 bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. *J Clin Oncol.*
999 2005;23(4):667–75.
- 1000 158. Fisher RI, Bernstein SH, Kahl BS, Djulbegovic B, Robertson MJ, de Vos S, Epner E,
1001 Krishnan A, Leonard JP, Lonial S, Stadtmauer EA, O'Connor OA, Shi H, Boral AL, Goy
1002 A. Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle
1003 cell lymphoma. *J Clin Oncol.* 2006;24(30):4867–74.
- 1004 159. Ruan J, Martin P, Furman RR, Lee SM, Cheung K, Vose JM, Lacasce A, Morrison J,
1005 Elstrom R, Ely S, Chadburn A, Cesarman E, Coleman M, Leonard JP. Bortezomib plus
1006 CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell
1007 lymphoma. *J Clin Oncol.* 2011;29(6):690–7.
- 1008 160. Kane RC, Dagher R, Farrell A, Ko C-W, Sridhara R, Justice R, Pazdur R. Bortezomib
1009 for the treatment of mantle cell lymphoma. *Clin Cancer Res.* 2007;13(18 Pt 1):5291–4.
- 1010 161. Crawford LJ, Walker B, Irvine AE. Proteasome inhibitors in cancer therapy. *J Cell*
1011 *Commun Signal.* 2011;5(2):101–10.
- 1012 162. McConkey DJ, Zhu K. Mechanisms of proteasome inhibitor action and resistance in
1013 cancer. *Drug Resist Updat.* 2008;11(4-5):164–79.
- 1014 163. Paoluzzi L, O'Connor OA. Mechanistic rationale and clinical evidence for the efficacy of
1015 proteasome inhibitors against indolent and mantle cell lymphomas. *BioDrugs.*
1016 2006;20(1):13–23.
- 1017 164. Pham LV, Tamayo AT, Yoshimura LC. Inhibition of constitutive NF- κ B activation in
1018 mantle cell lymphoma B cells leads to induction of cell cycle arrest and apoptosis. *J*
1019 *Immunol.* 2003;171(1):88–95.
- 1020 165. Milhollen MA, Traore T, Adams-Duffy J, Thomas MP, Berger AJ, Dang L, Dick LR,
1021 Garnsey JJ, Koenig E, Langston SP, Manfredi M, Narayanan U, Rolfe M, Staudt LM,
1022 Soucy TA, Yu J, Zhang J, Bolen JB, Smith PG. MLN4924, a NEDD8-activating enzyme
1023 inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of
1024 NF- κ B-dependent lymphoma. *Blood.* 2010;116(9):1515–23.
- 1025 166. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, Shovlin M, Jaffe

- 1026 ES, Janik JE, Staudt LM, Wilson WH. Differential efficacy of bortezomib plus
1027 chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. *Blood*.
1028 2009;113(24):6069–76.
- 1029 167. Di Bella N, Taetle R, Kolibaba K, Boyd T, Raju R, Barrera D, Cochran EW, Dien PY,
1030 Lyons R, Schlegel PJ, Vukelja SJ, Boston J, Boehm KA, Wang Y, Asmar L. Results of a
1031 phase 2 study of bortezomib in patients with relapsed or refractory indolent lymphoma.
1032 *Blood*. 2010;115(3):475–80.
- 1033 168. Ribrag V, Tilly H, Casasnovas O, Bosly A, Bouabdallah R, Delarue R, Boue F, Bron D,
1034 Feugier P, Haioun C, Offner F, Coiffier B. Efficacy and toxicity of two schedules of
1035 bortezomib in patients with recurrent or refractory follicular lymphoma: a randomised
1036 phase II trial from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). *Eur J*
1037 *Cancer*. 2013;49(4):904–10.
- 1038 169. O'Connor OA, Portlock C, Moskowitz C, Hamlin P, Straus D, Gerecitano J, Gonen M,
1039 Dumitrescu O, Sarasohn D, Butos J, Neylon E, Mac-Gregor Cortelli B, Blumel S, Evens
1040 AM, Zelenetz AD, Wright J, Cooper B, Winter J, Vose J. Time to treatment response in
1041 patients with follicular lymphoma treated with bortezomib is longer compared with other
1042 histologic subtypes. *Clin Cancer Res*. 2010;16(2):719–26. 164. Uziel O, Cohen O,
1043 Beery E, Nordenberg J, Lahav M. The effect of Bortezomib and Rapamycin on
1044 Telomerase Activity in Mantle Cell Lymphoma. *Transl Oncol*. 2014 Dec;7(6):741–51.
- 1045 170. Uziel O, Cohen O, Beery E, Nordenberg J, Lahav M. The effect of Bortezomib and
1046 Rapamycin on Telomerase Activity in Mantle Cell Lymphoma. *Transl Oncol*.
1047 2014;7(6):741–51.
- 1048 171. Kim JE, Yoon DH, Jang G, Lee DH, Kim S, Park C-S, Huh J, Kim WS, Park J, Lee JH,
1049 Lee SI, Suh C. A phase I/II study of bortezomib plus CHOP every 2 weeks (CHOP-14)
1050 in patients with advanced-stage diffuse large B-cell lymphomas. *Korean J Hematol*.
1051 2012 Mar;47(1):53–9.
- 1052 172. Mounier N, Ribrag V, Haioun C, Salles G, Golfier J, Ertault M, Ferme C, Briere J, Brice
1053 P, De Kerviler E, Gisselbrecht C. Abstract 8010: Efficacy and toxicity of two schedules
1054 of R-CHOP plus bortezomib in front-line B lymphoma patients: A randomized phase II
1055 trial from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). *J Clin Oncol*.
1056 2007;25(18S).
- 1057 173. Tobinai K, Ogura M, Maruyama D, Uchida T, Uike N, Choi I, Ishizawa K, Itoh K, Ando K,
1058 Taniwaki M, Shimada N, Kobayashi K. Phase I study of the oral mammalian target of
1059 rapamycin inhibitor everolimus (RAD001) in Japanese patients with relapsed or
1060 refractory non-Hodgkin lymphoma. *Int J Hematol*. 2010;92(4):563–70.
- 1061 174. Barnes JA, Jacobsen E, Feng Y, Freedman A, Hochberg EP, LaCasce AS, Armand P,
1062 Joyce R, Sohani AR, Rodig SJ, Neuberg D, Fisher DC, Abramson JS. Everolimus in
1063 combination with rituximab induces complete responses in heavily pretreated diffuse
1064 large B-cell lymphoma. *Haematologica*. 2013;98(4):615–9.

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1066

1067 Figure Legends**1068 Figure 1: mTOR signaling pathway**

1069 mTOR exists in two distinct complexes (mTORC1 and mTORC2) that are regulated separately
1070 and have distinct substrates. Whereas mTORC2 is regulated downstream of PI3K, mTORC1 is
1071 coordinately regulated by growth factor signals, nutrient availability (amino acids), and cellular
1072 energy status (ATP levels). The outputs of their downstream effectors coordinate processes
1073 required for cell growth including survival, inhibition of autophagy, protein translation and cell
1074 cycle progression.

1075

1076 Figure 2: Mechanisms of aberrant mTOR activation in B-cell malignancies

1077 Different proteins are amplified or activated (shown in red) in B-cell malignancies that result in
1078 increased mTOR activity. Where as the loss of the tumor suppressor PTEN (shown in blue)
1079 promotes mTOR activation. These mutations negate the normal constraints on mTOR activity
1080 to promote cancer cell proliferation.

1081

1082 Figure 3: Different effects of rapamycin and TOR-KI on mTOR activity

1083 Rapamycin (and all rapalogs) only partially inhibits mTOR activity. Rapamycin forms a complex
1084 with FKB12 to inhibit TORC1 activation of S6K activity and only partially reduces effects on
1085 4EBP. S6K normally negatively feeds back to inhibit the activation of PI3K. By suppressing
1086 S6K, rapamycin negates the feedback inhibition resulting in increased PI3K, TORC2 and AKT
1087 activation. Thus, survival signals from AKT highly active and 4EBP is partially active.
1088 Conversely, TOR-KIs suppresses all mTOR survival outputs.

1089

1090 Figure 4: Combination of targeting BCL-2 and mTOR

1091 (A) The BCL-2 family of proteins consists of pro- and anti-apoptotic members that interact
1092 antagonistically to determine cell fate. Survival signaling through AKT and mTOR increases the

1093 anti-apoptotic family members and decreases the pro-apoptotic members. (B) By inhibiting
1094 mTOR activity the balance is shifted where anti-apoptotic family members are reduced and pro-
1095 apoptotic members are increased. This reduces the capacity of anti-apoptotic proteins to
1096 sequester the pro-apoptotic proteins. Addition of ABT-199, a BCL-2 inhibitor, further shifts this
1097 balance to release the pro-apoptotic proteins and cause cancer killing.
1098

1

1 **Targeting mTOR for the treatment of B cell malignancies**

2

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16 Running head: mTOR inhibitors in B cell malignancies

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18 Keywords: mTOR, rapamycin, rapalogs, TOR-KIs, leukemia, lymphoma

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23 | Summary Abstract

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

39 | Introduction

40 | The mTOR Signaling Pathway

41 | *The mTOR Signaling Pathway*

42 | mTOR is a serine/threonine kinase that functions as a master regulator of cell growth,
43 | proliferation, metabolism, and survival. mTOR is active in two distinct multi-protein complexes
44 | (mTORC1 and mTORC2) that are characterized by the defining subunits RAPTOR and
45 | RICTOR respectively [1,2]. Each complex is differentially regulated and has a distinct set of
46 | substrates (Figure 1). Activation of mTORC2 activation is incompletely understood, but has
47 | recently been shown to be directly dependent on the generation of regulated by the levels of
48 | PI(3,4,5)P₃ produced by phosphoinositide 3-kinase (PI3K) [3]. Upon activation mTORC2

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

58 Upon activation, mTORC1 promotes key biosynthetic pathways including translation,
59 transcription, and lipogenesis, while suppressing apoptotic and autophagic processes
60 [11,12][8,9]. The most well-characterized downstream targets of mTORC1 ~~include are~~ the p70
61 ribosomal-S6 kinases (S6Ks), ~~which are critical for lipid and ribosome biogenesis pathways,~~ and
62 eukaryotic initiation factor 4E (eIF4E) ~~binding proteins (4E-BPs),~~ ~~Phosphorylation of S6Ks~~
63 ~~induces its activity, which is critical for lipid and ribosome biogenesis pathways and promotes~~
64 ~~translation via suppression of PDCD4 and activation of eIF4B [13,14]. In contrast,~~
65 ~~phosphorylation of 4E-BPs suppresses their ability to inhibit eIF4E, which promotes translation~~
66 ~~initiation which promotes translation of cap-bound mRNA transcripts (Figure 1). Whereas~~
67 ~~mTORC1 activates S6Ks directly, it activates eIF4E indirectly by suppressing the inhibitory~~
68 ~~function of eIF4E-binding proteins (4E-BPs) [15][10].~~ Together, these effectors ~~promote~~
69 ~~coordinately~~ increased protein synthesis ~~rates~~, a process whose dysregulation is a central
70 driving mechanism in cancer [16,17][11,12]. Importantly, hyper-activating mutations in mTOR
71 itself have been identified in many cancers ~~s and~~, further indicat~~esing~~ the importance of mTOR
72 activity to tumorigenesis [18][13].

73

74 *Evidence of mTOR activation in B-ALL and NHL*

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

87 Among NHL subtypes, activation of mTOR is consistently a reliable indicator of more
88 aggressive disease and poorer prognosis [26-30][21-25]. Similar to B-ALL, activation of mTOR
89 follows through direct mutations in key upstream pathways. In mantle cell lymphoma (MCL),
90 amplification of *PIK3CA* (the gene encoding the catalytic subunit of PI3K) and/or PTEN loss (the
91 negative regulator of PI3K activity) have been observed in a large fraction of primary tissue
92 samples [31][26]. In diffuse large B cell lymphoma (DLBCL), activation may be similarly
93 achieved via mutations in *PIK3CA* [32,33][27,28], or chronic B cell receptor activation [34][29].
94 In follicular lymphoma (FL), mTOR is aberrantly activated by way of PKC ζ or Syk kinases [35-
95 38][30-33]. Collectively, these data highlight the impact of elevated mTOR activity on patient
96 outcomes, and provide a solid rationale for the use of mTOR-targeted therapies in these B cell
97 malignancies.

98
99 **Rapalogs****Rapamycin and Rapalogs: partial mTORC1 inhibitors**

100 *Mechanism of action*

101 | Upon entry into a cell, rapamycin binds to FKBP12, forming a complex that potently and
102 | selectively suppresses mTORC1 kinase activity by limiting substrate access to the active site
103 | [39,40][34,35]. Importantly, the rapamycin-FKBP12 complex cannot bind to mTORC2
104 | [2,41][2,36], though in some cases, prolonged exposure may limit the assembly of mTORC2
105 | [42][37]. In this manner, rapamycin behaves as a highly potent and selective inhibitor of
106 | mTORC1 (Figure 3). However, poor solubility and pharmacokinetics spurred the development of
107 | rapamycin analogs (termed rapalogs) for oral dosing in cancer patients [43][38]. Most notable
108 | among these rapalogs are temsirolimus (CCI-779, Wyeth Pharmaceuticals [44][39]), everolimus
109 | (RAD001, Novartis Pharmaceuticals [45][40]), and ridaforolimus (AP23573, Merck and ARIAD
110 | Pharmaceuticals [46][41]).

111

112 | *Rapamycin and Rapalogs in B-ALL*

113 | Early testing with rapamycin unveiled potent anti-proliferative efficacy in several preclinical
114 | models of ALL. In an E μ -RET model of murine B-pre ALL, rapamycin as a single agent potently
115 | inhibited proliferation of leukemia cells both *in vitro* and *in vivo* [47,48][42,43]. Similar efficacy
116 | was later observed in models of Ph⁺ B-ALL [49,50][44,45], as well as in Ph-like B-ALL, driven
117 | by JAK pathway mutations or CRLF2 rearrangement [51][46]. Rapalogs also demonstrated
118 | marked preclinical efficacy in primary human ALL samples grown *in vitro* or in xenograft models
119 | [50-52]. Notably, rapamycin demonstrated single-agent cytotoxicity in primary pediatric ALL
120 | samples, and sensitized cells to doxorubicin *in vitro* [52][47]. Both everolimus and temsirolimus
121 | have shown similar efficacy in xenograft models of adult and pediatric primary human ALL as
122 | single agents [53][48] and in combination with chemotherapy [54,55][49,50].

123 | Clinically, rapamycin as a single agent exhibited no dose-limiting toxicities, but had
124 | lackluster efficacy compared to standard chemotherapeutic options (Table 1). In an early trial,
125 | rapamycin yielded stable disease in only three out of nine pediatric patients with relapsed ALL
126 | [56][54]. As a result, several trials have been launched to determine whether rapalogs can

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

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

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

154

155 *Rapalogs in NHL*

156 Similar to B-ALL, preclinical testing of rapalogs in NHL revealed promising cytostatic effects
157 both *in vitro* and *in vivo*, yet clinical responses were limited in most contexts. For example, in
158 MCL, FL, and DLBCL, rapamycin potently suppressed the proliferation of cell lines and primary
159 patient cells *in vitro* [62-66][56-60]. However, the clinical use of rapalogs has only made
160 progress in MCL where ~~chemotherapeutic~~ responses to standard chemotherapies are limited
161 (Table 1). In phase II trials of relapsed MCL, single agent administration of either temsirolimus
162 or ridaforolimus yielded overall response rates (ORR) of 38% [67][64] and 33% [68][62]
163 respectively. Notably, a subsequent phase II trial using a 10-fold lower dose of temsirolimus
164 revealed that similar responses could be obtained with lower toxicity [69][63]. Based on these
165 results, a randomized phase III trial was conducted. Strikingly, the ~~overall response rates~~ORR
166 and progression free survival were significantly higher in patients treated with temsirolimus
167 compared to investigator's choice agent. These results ultimately led to approval for
168 temsirolimus as a single agent therapy for relapsed/refractory MCL in Europe [70][64]. A
169 subsequent phase II trial has also been completed combining temsirolimus with rituximab in
170 relapsed/refractory MCL. Despite demonstrating higher response rates than single agent
171 temsirolimus, the combination was also associated with higher toxicities including
172 thrombocytopenia and neutropenia in a significant fraction of patients [71][65]. Rapalog
173 monotherapy has also elicited responses in a subset of patients with other NHL subtypes. In a
174 phase II trial of everolimus in relapsed lymphoma, the ORR in DLBCL was 30% (14/47) and
175 38% (3/8) in FL [72][66]. Similar results were seen with temsirolimus where the ORR was 28%
176 for DLBCL and 53% in FL [73][67]. While these studies highlight that rapalogs have some
177 activity, the availability of better therapeutic options in both DLBCL and FL has limited the

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

181

182 *Outlook:*

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

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

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

213

214 **TOR-KIs: complete mTORC1/2 inhibitors**

215 The timely development of mTOR kinase inhibitors (TOR-KIs) directly addressed the
216 biochemical disadvantages of rapalogs. By competing with ATP for binding to the mTOR active
217 site, not only do TOR-KIs more completely block mTORC1 substrate phosphorylation (namely
218 4E-BPs), but they also inhibit mTORC2 activity [87,88][81,82]. This results in reduced
219 phosphorylation of AKT at Ser473 (Figure 3), dampening the feedback activation of PI3K/AKT
220 that is known to limit rapalog efficacy [89-91][83-85]. It is important to note that by competing
221 with ATP, TOR-KIs are capable of inhibiting several kinases at higher doses, including the
222 structurally related protein, PI3K. Conversely, several compounds that are often used pre-
223 clinically as PI3K inhibitors (wortmannin, LY294002) directly inhibit mTORC1 and mTORC2 at
224 similar concentrations. Thus, it is important to fully understand the pharmacologic properties of
225 ATP-competitive mTOR and PI3K inhibitors when interpreting their preclinical and clinical
226 efficacy.

227 Several structurally distinct mTOR-selective inhibitors have been reported and tested in
228 models of B cell malignancies. Most notable among them are PP242 [88][82], Torin1 [87][84],
229 Ku-0063794 [92][86], AZD8055 [93][87], AZD2014 [93][87], MLN0128 (previously INK128

230 | [94][88], and CC-223 [95][89]. In preclinical testing, these TOR-KIs proved superior to rapalogs
231 | in terms of cytostatic and cytotoxic potential. For example, in a mouse model of AKT-driven
232 | lymphangiogenesis, PP242 strongly suppressed both 4E-BP1 phosphorylation and tumor
233 | growth compared to rapamycin [96][90]. These findings were also recapitulated *in vitro* using
234 | leukemia and DLBCL cell lines where TOR-KIs had a greatly improved biochemical effect on
235 | downstream 4E-BP phosphorylation [97-99][94-93].

236 | Despite the broader biochemical impact of TOR-KIs over rapalogs, whether complete mTOR
237 | kinase inhibition is sufficient to elicit cytotoxic responses is yet to be established. Two reports of
238 | structurally distinct TOR-KIs in B-ALL demonstrated that mTOR kinase inhibition was sufficient
239 | to induce apoptosis in B-ALL cell lines compared to rapamycin [100,101][94,95]. However, in
240 | both studies, apoptosis was only observed at doses of TOR-KI that greatly exceed what was
241 | needed to fully suppress mTOR kinase activity as measured by western blot. At lower doses
242 | that still fully suppress mTOR activity, our lab has found that both AZD8055 and MLN0128
243 | maintain a primarily cytostatic response profile (that is greater than rapalogs) [98,102-
244 | 104][92,96-98]. Notably, low doses of PP242 were sufficient to kill murine bone marrow cells
245 | immortalized by p190-BCR-ABL [99][93], suggesting that fully transformed B-ALL cells with
246 | additional oncogenic lesions may respond differently to mTOR inhibition. Thus, it remains
247 | unclear whether TOR-KIs will be effective in B-ALL or NHL as single agents at doses that are
248 | highly selective for mTOR kinase activity.

249 | Early clinical trials have suggested that while TOR-KIs are more effective than rapalogs at
250 | suppressing tumor growth, they may also be less tolerable [78][72]. A single agent tolerability
251 | test of AZD2014 showed dose-limiting toxicities that were similar to rapalogs including mucositis
252 | and fatigue [105][99]. Both CC-223 and MLN0128, also presented similar toxicities, ~~but in~~
253 | ~~addition to~~ hyperglycemia, ~~also occurred and~~ necessitated ~~teding~~ close monitoring of patient blood
254 | [106,107][100,101]. Several additional clinical trials are currently in progress to address the
255 | efficacy and tolerability of TOR-KIs and are summarized in **Table 2**. However, a key question is

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

262

263 **Emerging Combinations with mTOR Inhibitors:**

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

272

273 *Combinations targeting resistance mechanisms*

274 Targeting parallel and downstream pathways

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

282 resistance mechanisms, the addition of MAPK inhibitors and RTK inhibitors have demonstrated
283 significantly more efficacy in combination with both rapalogs and TOR-KIs in preclinical settings
284 [\[80,109,110\]](#)[\[74,103,104\]](#). However, in other instances, resistance to mTOR inhibition may be a
285 result of sustained downstream effector activity, particularly cap-dependent translation. For
286 example, our laboratory has noted resistance to TOR-KIs in DLBCL cell lines lacking expression
287 of 4E-BPs [\[98\]](#)[\[92\]](#) or, and over-expression of eIF4E [\[111\]](#)[\[105\]](#).
288 Furthermore, recent evidence has indicated that PIM and MNK kinases can maintain cap-
289 dependent translation downstream of mTORC1 inhibition [\[112\]](#)[\[106\]](#) (Figure 4). In these
290 situations, targeting cap-dependent translation indirectly using combinations of PIM or MNK
291 inhibitors with TOR-KIs has shown cytotoxic activity in AML cell lines [\[113,114\]](#)[\[107,108\]](#) as well
292 as in cutaneous T cell lymphoma cell lines *in vitro* [\[115\]](#)[\[109\]](#). Additional work is required to
293 evaluate the potential of directly targeting the cap-dependent translation initiation machinery. It
294 is likely that other mechanisms of resistance will arise as our experience with mTOR inhibitors
295 increases, and these may ultimately support the study of additional combinations.

296 While clinical data regarding the efficacy of these combinations in B cell malignancies has
297 not reached maturity, similar combinations have been successfully deployed in non-hematologic
298 malignancies. For example, inhibition of the upstream tyrosine kinase, HER2, significantly
299 improved the efficacy of mTORC1/2 inhibition in patients with refractory breast cancer
300 compared to single agent treatment [\[116\]](#)[\[140\]](#). Similarly, combinations of PI3K/AKT/mTOR and
301 Ras/MAPK/ERK pathway inhibition yielded improved response rates in patients with advanced
302 refractory solid tumors, but did so at the cost of significantly higher toxicities [\[117\]](#)[\[144\]](#).
303 Collectively these studies highlight the potential of using mTOR inhibitors in combination with
304 agents targeting known resistance pathways to mTOR inhibition, or as an adjuvant therapy to
305 augment the effects of other rational targeted therapies. However, it will be important to
306 determine whether these combinations targeting multiple key survival pathways will remain
307 selective for cancer cells as toxicity will be a major concern.

308

309 Targeting apoptosis

310 Another straightforward approach to directly enhancing the apoptotic potential of mTOR
311 inhibition is to target the pro-survival BCL-2 family proteins. Apoptosis is regulated through
312 dynamic and competitive binding interactions between anti-apoptotic proteins (e.g. BCL-2, BCL-
313 XL, BCL-w, and MCL-1) and pro-apoptotic sensitizers (e.g. BAD, PUMA, and NOXA), activators
314 (e.g. BIM and BID), and effectors (BAX and BAK) (Figure 45A). While mTOR inhibition is known
315 to suppress survival signaling through both mTORC1 (e.g. MCL-1 expression [96][99]) and AKT
316 (e.g. inhibition of BAD and down-regulation of BIM [118,119][442,443]), TOR-KIs are insufficient
317 to induce apoptosis through this pathway. Thus, a simple approach would be to use antagonists
318 of the pro-survival proteins to disrupt their binding capacity, and subsequently lower the
319 threshold for BIM to activate BAX/BAK-mediated MOMP and apoptosis [120][444].

320 ABT-737, and its orally bioavailable analog, ABT-263, represent the most potent and
321 selective small molecule inhibitors of BCL-2 and BCL-X_L. Both of these compounds
322 demonstrated remarkable cytotoxic potential that was significantly enhanced when combined
323 with mTOR inhibitors in DLBCL [121][445], FL [122][446], AML [123][447], and B-ALL
324 [124][448]. However, due to on-target toxicity associated with BCL-X_L inhibition [125][449], a
325 more promising clinical candidate is ABT-199 [126][420]. ABT-199 is a selective inhibitor of
326 BCL-2, and has elicited substantial clinical responses in patients with CLL as a single agent
327 [127][424], leading to its designation as a breakthrough therapy for CLL patients with a 17p
328 deletion (p53). Importantly, we and others have recently reported that ABT-199 synergizes with
329 mTOR inhibition comparably to dual BCL-2/BCL-X_L inhibitors [104,128][98,422], suggesting that
330 the rationale established using first generation BCL-2 antagonists will hold true for ABT-199.
331 However, a key concern is whether the addition of TOR-KIs to BCL-2 antagonists will enhance
332 its toxicity towards non-cancer cells. In an effort to address this question, our lab has recently
333 demonstrated that the combination does not synergize to kill peripheral blood mononuclear cells

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

336

337

338 | *mTOR inhibition as an adjuvant*

339 | Targeting oncogenic drivers

340 | In contrast to targeting resistance mechanisms, others have found that combining

341 | oncogene-targeted therapies with mTOR inhibition also holds promise in B cell malignancies.

342 | For example, in Ph+ B-ALL driven by the BCR-ABL translocation, both rapamycin and PP242

343 | strongly synergized with imatinib to suppress leukemia growth [99][93]. Similarly, in

344 | myeloproliferative disorders characterized by JAK2 mutations, combinations of TOR-KIs or

345 | rapalogs with JAK2 inhibitors synergistically killed cells whereas single-agent treatments were

346 | primarily cytostatic [129,130][123,124]. In the activated B cell like (ABC) subtype of DLBCL,

347 | which is driven by sustained activation of the B cell receptor (BCR) [34][29], inhibition of the

348 | downstream kinase, Bruton's tyrosine kinase (BTK), also synergized strongly with

349 | PI3K/AKT/mTOR inhibitors [131][125]. However, the limitations of this approach are also

350 | ~~recently~~ becoming apparent. In particular, the germinal center B cell-like (GCB) DLBCL subtype

351 | is unresponsive to combinations of BTK and mTOR inhibitors, likely because BCR activation is

352 | not an oncogenic driver in this setting [132][126]. More alarmingly, in some cases the addition of

353 | mTOR inhibitors may antagonize the effects of other agents, either through suppression of

354 | proliferation or through induction of autophagy [133,134][127,128]. Studies like these serve as

355 | powerful reminders that a sound biological understanding supporting the use of these

356 | combinations must precede their clinical use.

357

358 | Targeting histone deacetylases (HDACs)

359 HDAC inhibitors are another promising class of drug that may benefit from the addition of
360 mTOR inhibitors. In addition to modulating histone function and gene expression, HDACs also
361 regulate the activity of non-histone proteins with relevance to B cell cancers (e.g. STAT, Hsp90,
362 and FOXO) [135-138][129-132]. Importantly, mutations in genes regulating protein acetylation
363 have been described in both B-ALL and NHL. For example, mutations in the CREBBP histone
364 acetyltransferase (HAT) domain have been identified in a subset of patients with relapsed
365 pediatric B-ALL where it may confer glucocorticoid resistance [139][133]. Similar mutations in
366 HAT activity were identified as frequent mutations in both FL and DLBCL where their
367 inactivation promotes aberrant up-regulation of BCL-6, a protein known to promote B cell
368 malignancies [140-142][134-136]. Given the pervasive importance of protein acetylation, it is
369 unsurprising that HDAC inhibitors have elicited promising responses in various leukemias and
370 lymphomas. For example, in lymphomas with a t(14;18) translocation, HDAC inhibitors were
371 shown to markedly reduce expression of BCL-2 leading to apoptosis [143][137]. In other
372 contexts, HDAC inhibition can induce mitochondrial apoptosis via epigenetic regulation of other
373 BCL-2 family proteins [144,145][138,139], production of reactive oxygen species and ceramide
374 [146][140], or activation of death receptors [147][144]. Potent anti-proliferative effects have also
375 been described [145,148][139,142]. Importantly, recent evidence has suggested that the
376 addition of mTOR inhibition may augment the effects of HDAC inhibitors. For example, our lab
377 has recently identified synergy between HDAC inhibitors and TOR-KIs in B-ALL cell lines and
378 primary patient samples [103][97]. Also, both temsirolimus and everolimus have demonstrated
379 synergistic anti-proliferative and apoptotic effects when combined with the HDAC inhibitors in
380 MCL [149,150][143,144]. In DLBCL, combining HDAC inhibitors with rapalogs or TOR-KIs also
381 synergistically induced apoptosis [65,151][59,145]. While there is still debate as to the exact
382 mechanism of synergy, it is clear that in a preclinical setting, this combination has marked
383 potential in B cell malignancies. However, in a phase I trial combining panobinostat and
384 everolimus in relapsed/refractory lymphoma, the combination yielded ORRs similar to

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

389

390 | Targeting the proteasome

391 | Another class of inhibitors that has shown promise in B cell malignancies are proteasome
392 | inhibitors [153][147]. Interestingly, even across several cancer subtypes, these inhibitors have
393 | been most promising in B cell malignancies [154-159][148-153], as evidenced by the FDA
394 | approval for bortezomib in both relapsed MCL and multiple myeloma [160][154]. By suppressing
395 | degradation of proteins, these inhibitors induce a plethora of cellular responses leading to anti-
396 | proliferative and pro-apoptotic effects [161,162][155,156]. Most notable among these effects are
397 | its ability to suppress NF-κB activity and modulate expression of BCL-2 family proteins [162-
398 | 164][156-158], which provides the basis for single agent bortezomib efficacy in ABC-DLBCL
399 | [165,166][159,160]. However, in other B cell malignancies, single agent proteasome inhibition is
400 | not as effective [167-169][161-163]. While preclinical data has suggested some synergy
401 | between rapalogs and bortezomib [150,170][144,164], whether combined proteasome and
402 | mTOR inhibition will have generalizable efficacy is still unclear. A major clinical concern with
403 | bortezomib is neurological toxicity [171,172][165,166], and while dose management may
404 | alleviate some risks, it is unclear what effects the addition of mTOR inhibitors may have on
405 | patient outcomes.

406

407 | **Outlook**

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

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

428

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433

434

435 **Conflict of Interest Statement**

436 [All authors have completed the Unified Competing Interest form at](#)

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

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441 **References**

- 442 1. Kim D-H, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P,
443 Sabatini DM. mTOR Interacts with Raptor to Form a Nutrient-Sensitive Complex that
444 Signals to the Cell Growth Machinery. *Cell*. 2002;110(2):163–75.
- 445 2. Sarbassov DD, Ali SM, Kim D-H, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst
446 P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-
447 insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol*.
448 2004;14(14):1296–302.
- 449 3. Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, Wang B, Blenis J, Cantley LC, Toker
450 A, Su B, Wei W. PtdIns(3,4,5)P3-Dependent Activation of the mTORC2 Kinase
451 Complex. *Cancer Discov*. 2015;5(11):1194–209.
- 452 4. Sarbassov DD. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR
453 Complex. *Science*. 2005;307(5712):1098–101.
- 454 5. Marte BM, Downward J. PKB/Akt: connecting phosphoinositide 3-kinase to cell survival
455 and beyond. *Trends Biochem Sci*. 1997;22(9):355–8.
- 456 6. Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD,
457 Chantranupong L, Comb W, Wang T, Bar-Peled L, Zoncu R, Straub C, Kim C, Park J,
458 Sabatini BL, Sabatini DM. Lysosomal amino acid transporter SLC38A9 signals arginine
459 sufficiency to mTORC1. *Science*. 2015;347(6218):188–94.
- 460 7. Rebsamen M, Pochini L, Stasyk T, de Araújo MEG, Galluccio M, Kandasamy RK,
461 Snijder B, Fauster A, Rudashevskaya EL, Bruckner M, Scorzoni S, Filipek PA, Huber
462 KVM, Bigenzahn JW, Heinz LX, Kraft C, Bennett KL, Indiveri C, Huber LA, Superti-
463 Furga G. SLC38A9 is a component of the lysosomal amino acid sensing machinery that
464 controls mTORC1. *Nature*. 2015;519(7544):477–81.
- 465 8. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer,
466 diabetes and ageing. *Nat Rev Mol Cell Biol*. 2010;12(1):21–35.
- 467 9. Laplante M, Sabatini DM. mTOR Signaling in Growth Control and Disease. *Cell*.
468 2012;149(2):274–93.
- 469 10. Hay N. Upstream and downstream of mTOR. *Genes Dev*. 2004;18(16):1926–45.
- 470 11. Kamada Y, Yoshino K-I, Kondo C, Kawamata T, Oshiro N, Yonezawa K, Ohsumi Y. Tor
471 directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol*.
472 2010;30(4):1049–58.
- 473 12. Chang Y-Y, Neufeld TP. An Atg1/Atg13 complex with multiple roles in TOR-mediated
474 autophagy regulation. *Mol Biol Cell*. 2009;20(7):2004–14.
- 475 13. Schmid T, Jansen AP, Baker AR, Hegamyer G, Hagan JP, Colburn NH. Translation
476 inhibitor Pdc4 is targeted for degradation during tumor promotion. *Cancer Res*.
477 2008;68(5):1254–60.

- 478 14. Shahbazian D, Roux PP, Mieulet V, Cohen MS, Raught B, Taunton J, Hershey JWB,
479 Blenis J, Pende M, Sonenberg N. The mTOR/PI3K and MAPK pathways converge on
480 eIF4B to control its phosphorylation and activity. *EMBO J.* 2006;25(12):2781–91.
- 481 15. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF,
482 Aebersold R, Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step
483 mechanism. *Genes Dev.* 1999;13(11):1422–37.
- 484 16. Mamane Y, Petroulakis E, LeBacquer O, Sonenberg N. mTOR, translation initiation and
485 cancer. *Oncogene.* 2006;25(48):6416–22.
- 486 17. Bader AG, Kang S, Zhao L, Vogt PK. Oncogenic PI3K deregulates transcription and
487 translation. *Nat Rev Cancer.* 2005;5(12):921–9.
- 488 18. Grabiner BC, Nardi V, Birsoy K, Possemato R, Shen K, Sinha S, Jordan A, Beck AH,
489 Sabatini DM. A Diverse Array of Cancer-Associated MTOR Mutations Are
490 Hyperactivating and Can Predict Rapamycin Sensitivity. *Cancer Discov.* 2014;4(5):554–
491 63.
- 492 19. Pfeifer H, Wassmann B, Pavlova A, Wunderle L, Oldenburg J, Binckebanck A, Lange T,
493 Hochhaus A, Wystub S, Brück P, Hoelzer D, Ottmann OG. Kinase domain mutations of
494 BCR-ABL frequently precede imatinib-based therapy and give rise to relapse in patients
495 with de novo Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood.*
496 2007;110(2):727–34.
- 497 20. Crist W, Carroll A, Shuster J, Jackson J, Head D, Borowitz M, Behm F, Link M, Steuber
498 P, Ragab A. Philadelphia chromosome positive childhood acute lymphoblastic
499 leukemia: clinical and cytogenetic characteristics and treatment outcome. A Pediatric
500 Oncology Group study. *Blood.* 1990;76(3):489–94.
- 501 21. Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, Chen S-C, Payne-Turner D,
502 Churchman ML, Harvey RC, Chen X, Kasap C, Yan C, Becksfort J, Finney RP,
503 Teachey DT, Maude SL, Tse K, Moore R, Jones S, Mungall K, Birol I, Edmonson MN,
504 Hu Y, Buetow KE, Chen I-M, Carroll WL, Wei L, Ma J, Kleppe M, Levine RL, Garcia-
505 Manero G, Larsen E, Shah NP, Devidas M, Reaman G, Smith M, Paugh SW, Evans
506 WE, Grupp SA, Jeha S, Pui C-H, Gerhard DS, Downing JR, Willman CL, Loh M,
507 Hunger SP, Marra MA, Mullighan CG. Genetic Alterations Activating Kinase and
508 Cytokine Receptor Signaling in High-Risk Acute Lymphoblastic Leukemia. *Cancer Cell.*
509 2012;22(2):153–66.
- 510 22. Tasian SK, Doral MY, Borowitz MJ, Wood BL, Chen I-M, Harvey RC, Gastier-Foster
511 JM, Willman CL, Hunger SP, Mullighan CG, Loh ML. Aberrant STAT5 and PI3K/mTOR
512 pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic
513 leukemia. *Blood.* 2012;120(4):833–42.
- 514 23. Morishita N, Tsukahara H, Chayama K, Ishida T, Washio K, Miyamura T, Yamashita N,
515 Oda M, Morishima T. Activation of Akt is associated with poor prognosis and
516 chemotherapeutic resistance in pediatric B-precursor acute lymphoblastic leukemia.
517 *Pediatr Blood Cancer.* 2011;59(1):83–9.
- 518 24. Gomes AM, Soares MVD, Ribeiro P, Caldas J, Póvoa V, Martins LR, Melão A, Serra-

- 519 Caetano A, de Sousa AB, Lacerda JF, Barata JT. Adult B-cell acute lymphoblastic
520 leukemia cells display decreased PTEN activity and constitutive hyperactivation of
521 PI3K/Akt pathway despite high PTEN protein levels. *Haematologica*. 2014;99(6):1062–
522 8.
- 523 25. Nemes K, Sebestyén A, Márk Á, Hajdu M, Kenessey I, Sticz T, Nagy E, Barna G,
524 Váradi Z, Kovács G, Kopper L, Csóka M. Mammalian target of rapamycin (mTOR)
525 activity dependent phospho-protein expression in childhood acute lymphoblastic
526 leukemia (ALL). Bendall L, editor. *PLoS One*. 2013;8(4):e59335.
- 527 26. Peponi E, Drakos E, Reyes G, Leventaki V, Rassidakis GZ, Medeiros LJ. Activation of
528 mammalian target of rapamycin signaling promotes cell cycle progression and protects
529 cells from apoptosis in mantle cell lymphoma. *American J Pathol*. 2006;169(6):2171–
530 80.
- 531 27. Rudelius M, Pittaluga S, Nishizuka S, Pham THT, Fend F, Jaffe ES, Quintanilla-
532 Martinez L, Raffeld M. Constitutive activation of Akt contributes to the pathogenesis and
533 survival of mantle cell lymphoma. *Blood*. 2006;108(5):1668–76.
- 534 28. Uddin S, Hussain AR, Siraj AK, Manogaran PS, Al-Jomah NA, Moorji A, Atizado V, Al-
535 Dayel F, Belgaumi A, El-Solh H, Ezzat A, Bavi P, Al-Kuraya KS. Role of
536 phosphatidylinositol 3'-kinase/AKT pathway in diffuse large B-cell lymphoma survival.
537 *Blood*. 2006;108(13):4178–86.
- 538 29. Hasselblom S, Hansson U, Olsson M, Torén L, Bergström A, Nilsson-Ehle H,
539 Andersson P-O. High immunohistochemical expression of p-AKT predicts inferior
540 survival in patients with diffuse large B-cell lymphoma treated with
541 immunochemotherapy. *Br J Haematol*. 2010;149(4):560–8.
- 542 30. Yu B-H, Zhou X-Y, Xiao X-Y, Yan S-Y, Qin T, Shi D-R. [Activation and clinicopathologic
543 significance of AKT/mTOR signaling pathway in diffuse large B-cell lymphoma].
544 *Zhonghua Bing Li Xue Za Zhi*. 2009;38(1):35–41.
- 545 31. Psyri A, Papageorgiou S, Liakata E, Scorilas A, Rontogianni D, Kontos CK, Argyriou P,
546 Pectasides D, Harhalakis N, Pappa V, Kolialexi A, Economopoulou C, Kontsioti F,
547 Maratou E, Dimitriadis G, Economopoulou P, Economopoulos T. Phosphatidylinositol
548 3'-kinase catalytic subunit alpha gene amplification contributes to the pathogenesis of
549 mantle cell lymphoma. *Clin Cancer Res*. 2009;15(18):5724–32.
- 550 32. Baohua Y, Xiaoyan Z, Tiecheng Z, Tao Q, Daren S. Mutations of the PIK3CA gene in
551 diffuse large B cell lymphoma. *Diagn Mol Pathol*. 2008;17(3):159–65.
- 552 33. Abubaker J, Bavi PP, Al-harbi S, Siraj AK, Al-Dayel F, Uddin S, Al-Kuraya K. PIK3CA
553 mutations are mutually exclusive with PTEN loss in diffuse large B-cell lymphoma.
554 *Leukemia*. 2007;21(11):2368–70.
- 555 34. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, Kohlhammer H, Lamy
556 L, Zhao H, Yang Y, Xu W, Shaffer AL, Wright G, Xiao W, Powell J, Jiang J-K, Thomas
557 CJ, Rosenwald A, Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Johnson
558 NA, Rimsza LM, Campo E, Jaffe ES, Wilson WH, Delabie J, Smeland EB, Fisher RI,
559 Braziel RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Pierce SK, Staudt LM.

- 560 Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*.
561 2010;463(7277):88–92.
- 562 35. Leseux L, Laurent G, Laurent C, Rigo M, Blanc A, Olive D, Bezombes C. PKC zeta
563 mTOR pathway: a new target for rituximab therapy in follicular lymphoma. *Blood*.
564 2008;111(1):285–91.
- 565 36. Fruchon S, Kheirallah S, Saati Al T, Ysebaert L, Laurent C, Leseux L, Fournié JJ,
566 Laurent G, Bezombes C. Involvement of the Syk-mTOR pathway in follicular lymphoma
567 cell invasion and angiogenesis. *Leukemia*. 2012;26(4):795–805.
- 568 37. Leseux L, Hamdi SM, Saati al T, Capilla F, Récher C, Laurent G, Bezombes C. Syk-
569 dependent mTOR activation in follicular lymphoma cells. *Blood*. 2006;108(13):4156–62.
- 570 38. Gulmann C, Espina V, Petricoin E, Longo DL, Santi M, Knutsen T, Raffeld M, Jaffe ES,
571 Liotta LA, Feldman AL. Proteomic analysis of apoptotic pathways reveals prognostic
572 factors in follicular lymphoma. *Clin Cancer Res*. 2005;11(16):5847–55.
- 573 39. Choi J, Chen J, Schreiber SL, Clardy J. Structure of the FKBP12-rapamycin complex
574 interacting with the binding domain of human FRAP. *Science*. 1996;273(5272):239–42.
- 575 40. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP. mTOR kinase
576 structure, mechanism and regulation. *Nature*. 2013;497(7448):217–23.
- 577 41. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN. Mammalian TOR
578 complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*.
579 2004;6(11):1122–8.
- 580 42. Sarbassov DD, Ali SM, Sengupta S, Sheen J-H, Hsu PP, Bagley AF, Markhard AL,
581 Sabatini DM. Prolonged Rapamycin Treatment Inhibits mTORC2 Assembly and
582 Akt/PKB. *Mol Cell*. 2006;22(2):159–68.
- 583 43. Li J, Kim SG, Blenis J. Rapamycin: one drug, many effects. *Cell Metab*.
584 2014;19(3):373–9.
- 585 44. Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. *Clin Cancer Res*.
586 2008;14(5):1286–90.
- 587 45. Gabardi S, Baroletti SA. Everolimus: a proliferation signal inhibitor with clinical
588 applications in organ transplantation, oncology, and cardiology. *Pharmacotherapy*.
589 2010;30(10):1044–56.
- 590 46. Mita M, Sankhala K, Abdel-Karim I, Mita A, Giles F. Deforolimus (AP23573) a novel
591 mTOR inhibitor in clinical development. *Expert Opin Investig Drugs*. 2008;17(12):1947–
592 54.
- 593 47. Brown VI, Fang J, Alcorn K, Barr R, Kim JM, Wasserman R, Grupp SA. Rapamycin is
594 active against B-precursor leukemia in vitro and in vivo, an effect that is modulated by
595 IL-7-mediated signaling. *Proc Natl Acad Sci U S A*. 2003;100(25):15113–8.
- 596 48. Brown VI, Hulitt J, Fish J, Sheen C, Bruno M, Xu Q, Carroll M, Fang J, Teachey D,

- 597 Grupp SA. Thymic stromal-derived lymphopoietin induces proliferation of pre-B
598 leukemia and antagonizes mTOR inhibitors, suggesting a role for interleukin-7/Ralpha
599 signaling. *Cancer Res.* 2007;67(20):9963–70.
- 600 49. Kharas MG, Deane JA, Wong S, O'Bosky KR, Rosenberg N, Witte ON, Fruman DA.
601 Phosphoinositide 3-kinase signaling is essential for ABL oncogene-mediated
602 transformation of B-lineage cells. *Blood.* 2004;103(11):4268–75.
- 603 50. Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, Fruman DA.
604 Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR
605 inhibitor prevents expansion of human BCR-ABL+ leukemia cells. *J Clin Invest.*
606 2008;118(9):3038–50.
- 607 51. Maude SL, Tasian SK, Vincent T, Hall JW, Sheen C, Roberts KG, Seif AE, Barrett DM,
608 Chen I-M, Collins JR, Mullighan CG, Hunger SP, Harvey RC, Willman CL, Fridman JS,
609 Loh ML, Grupp SA, Teachey DT. Targeting JAK1/2 and mTOR in murine xenograft
610 models of Ph-like acute lymphoblastic leukemia. *Blood.* 2012;120(17):3510–8.
- 611 52. Avellino R, Romano S, Parasole R, Bisogni R, Lamberti A, Poggi V, Venuta S, Romano
612 MF. Rapamycin stimulates apoptosis of childhood acute lymphoblastic leukemia cells.
613 *Blood.* 2005;106(4):1400–6.
- 614 53. Teachey DT, Obzut DA, Cooperman J, Fang J, Carroll M, Choi JK, Houghton PJ, Brown
615 VI, Grupp SA. The mTOR inhibitor CCI-779 induces apoptosis and inhibits growth in
616 preclinical models of primary adult human ALL. *Blood.* 2006;107(3):1149–55.
- 617 54. Teachey DT, Sheen C, Hall J, Ryan T, Brown VI, Fish J, Reid GSD, Seif AE, Norris R,
618 Chang YJ, Carroll M, Grupp SA. mTOR inhibitors are synergistic with methotrexate: an
619 effective combination to treat acute lymphoblastic leukemia. *Blood.* 2008;112(5):2020–
620 3.
- 621 55. Crazzolara R, Cisterne A, Thien M, Hewson J, Baraz R, Bradstock KF, Bendall LJ.
622 Potentiating effects of RAD001 (Everolimus) on vincristine therapy in childhood acute
623 lymphoblastic leukemia. *Blood.* 2009;113(14):3297–306.
- 624 56. Rheingold SR, Sacks N, Chang YJ, Brown VI, Teachey DT, Lange BJ, Grupp SA. A
625 Phase I Trial of Sirolimus (Rapamycin) in Pediatric Patients with Relapsed/Refractory
626 Leukemia. 2007;110(11):2834.
- 627 57. Rheingold SR, Whitlock JA, Tasian SK, Teachey DT, Borowitz MJ, Liu X, Ahern CH,
628 Minard C, Fox E, Weigel B, Blaney S. Temsirolimus and intensive re-induction
629 chemotherapy for 2nd or greater relapse of acute lymphoblastic leukemia (ALL): A
630 Children's Oncology Group study. 2015 ASCO Annual Meeting. Chicago, IL.
- 631 58. Daver N, Bumber Y, Kantarjian H, Ravandi F, Cortes J, Rytting ME, Kawedia JD,
632 Basnett J, Culotta KS, Zeng Z, Lu H, Richie MA, Garris R, Xiao L, Liu W, Baggerly KA,
633 Jabbour E, O'Brien S, Burger J, Bendall LJ, Thomas D, Konopleva M. A Phase I/II
634 Study of the mTOR Inhibitor Everolimus in Combination with HyperCVAD
635 Chemotherapy in Patients with Relapsed/Refractory Acute Lymphoblastic Leukemia.
636 *Clin Cancer Res.* 2015;21(12):2704–14.

- 637 59. O'Brien S, Thomas D, Ravandi F, Faderl S, Cortes J, Borthakur G, Pierce S, Garcia-
638 Manero G, Kantarjian HM. Outcome of adults with acute lymphocytic leukemia after
639 second salvage therapy. *Cancer*. 2008;113(11):3186–91.
- 640 60. Gökbüget N, Stanze D, Beck J, Diedrich H, Horst H-A, Hüttmann A, Kobbe G, Kreuzer
641 K-A, Leimer L, Reichle A, Schaich M, Schwartz S, Serve H, Starck M, Stelljes M,
642 Stuhlmann R, Viardot A, Wendelin K, Freund M, Hoelzer D, German Multicenter Study
643 Group for Adult Acute Lymphoblastic Leukemia. Outcome of relapsed adult
644 lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic
645 factors, and performance of stem cell transplantation. *Blood*. 2012;120(10):2032–41.
- 646 61. Place AE, Pikman Y, Stevenson K, Harris MH, Cooper TM, Gore L, Hijjiya N, Loh ML,
647 Pauly M, Sulis M-L, Neuberg DS, Stegmaier K, Sallan SE, Silverman LB. Abstract 3765:
648 Phase Ib Trial of the mTOR Inhibitor Everolimus Given in Combination with Multiagent
649 Chemotherapy in Relapsed Acute Lymphoblastic Leukemia. 2015;126(23):3765.
- 650 62. Dal Col J, Zancai P, Terrin L, Guidoboni M, Ponzoni M, Pavan A, Spina M, Bergamin S,
651 Rizzo S, Tirelli U, De Rossi A, Doglioni C, Dolcetti R. Distinct functional significance of
652 Akt and mTOR constitutive activation in mantle cell lymphoma. *Blood*.
653 2008;111(10):5142–51.
- 654 63. Hipp S, Ringshausen I, Oelsner M, Bogner C, Peschel C, Decker T. Inhibition of the
655 mammalian target of rapamycin and the induction of cell cycle arrest in mantle cell
656 lymphoma cells. *Haematologica*. 2005;90(10):1433–4.
- 657 64. Gupta M, Dillon SR, Ziesmer SC, Feldman AL, Witzig TE, Ansell SM, Cerhan JR,
658 Novak AJ. A proliferation-inducing ligand mediates follicular lymphoma B-cell
659 proliferation and cyclin D1 expression through phosphatidylinositol 3-kinase-regulated
660 mammalian target of rapamycin activation. *Blood*. 2009;113(21):5206–16.
- 661 65. Gupta M, Ansell SM, Novak AJ, Kumar S, Kaufmann SH, Witzig TE. Inhibition of
662 histone deacetylase overcomes rapamycin-mediated resistance in diffuse large B-cell
663 lymphoma by inhibiting Akt signaling through mTORC2. *Blood*. 2009;114(14):2926–35.
- 664 66. Wanner K, Hipp S, Oelsner M, Ringshausen I, Bogner C, Peschel C, Decker T.
665 Mammalian target of rapamycin inhibition induces cell cycle arrest in diffuse large B cell
666 lymphoma (DLBCL) cells and sensitises DLBCL cells to rituximab. *Br J Haematol*.
667 2006;134(5):475–84.
- 668 67. Witzig TE, Geyer SM, Ghobrial I, Inwards DJ, Fonseca R, Kurtin P, Ansell SM, Luyun R,
669 Flynn PJ, Morton RF, Dakhil SR, Gross H, Kaufmann SH. Phase II trial of single-agent
670 temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol*.
671 2005;23(23):5347–56.
- 672 68. Rizzieri DA, Feldman E, DiPersio JF, Gabrail N, Stock W, Strair R, Rivera VM, Albitar
673 M, Bedrosian CL, Giles FJ. A phase 2 clinical trial of deforolimus (AP23573, MK-8669),
674 a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory
675 hematologic malignancies. *Clin Cancer Res*. 2008;14(9):2756–62.
- 676 69. Ansell SM, Inwards DJ, Rowland KM, Flynn PJ, Morton RF, Moore DF, Kaufmann SH,
677 Ghobrial I, Kurtin PJ, Maurer M, Allmer C, Witzig TE. Low-dose, single-agent

- 678 temsirolimus for relapsed mantle cell lymphoma: a phase 2 trial in the North Central
679 Cancer Treatment Group. *Cancer*. 2008;113(3):508–14.
- 680 70. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, Laurell A,
681 Offner F, Strahs A, Berkenblit A, Hanushevsky O, Clancy J, Hewes B, Moore L, Coiffier
682 B. Phase III Study to Evaluate Temsirolimus Compared With Investigator's Choice
683 Therapy for the Treatment of Relapsed or Refractory Mantle Cell Lymphoma. *J Clin
684 Oncol*. 2009;27(23):3822–9.
- 685 71. Ansell SM, Tang H, Kurtin PJ, Koenig PA, Inwards DJ, Shah K, Ziesmer SC, Feldman
686 AL, Rao R, Gupta M, Erlichman C, Witzig TE. Temsirolimus and rituximab in patients
687 with relapsed or refractory mantle cell lymphoma: a phase 2 study. *Lancet Oncol*.
688 2011;12(4):361–8.
- 689 72. Witzig TE, Reeder CB, LaPlant BR, Gupta M, Johnston PB, Micallef IN, Porrata LF,
690 Ansell SM, Colgan JP, Jacobsen ED, Ghobrial IM, Habermann TM. A phase II trial of
691 the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. *Leukemia*.
692 2011;25(2):341–7.
- 693 73. Smith SM, van Besien K, Karrison T, Dancey J, McLaughlin P, Younes A, Smith S, Stiff
694 P, Lester E, Modi S, Doyle LA, Vokes EE, Pro B. Temsirolimus Has Activity in Non-
695 Mantle Cell Non-Hodgkin's Lymphoma Subtypes: The University of Chicago Phase II
696 Consortium. *J Clin Oncol*. 2010;28(31):4740–6.
- 697 74. Dilling MB, Dias P, Shapiro DN, Germain GS, Johnson RK, Houghton PJ. Rapamycin
698 selectively inhibits the growth of childhood rhabdomyosarcoma cells through inhibition
699 of signaling via the type I insulin-like growth factor receptor. *Cancer Res*.
700 1994;54(4):903–7.
- 701 75. Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmithorst
702 VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN. Sirolimus for angiomyolipoma in
703 tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med*.
704 2008;358(2):140–51.
- 705 76. Marsh DJ, Trahair TN, Martin JL, Chee WY, Walker J, Kirk EP, Baxter RC, Marshall
706 GM. Rapamycin treatment for a child with germline PTEN mutation. *Nat Clin Pract
707 Oncol*. 2008;5(6):357–61.
- 708 77. Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E,
709 Sauter M, Nonomura N, Brakemeier S, de Vries PJ, Whittmore VH, Chen D, Sahmoud
710 T, Shah G, Lincy J, Lebwohl D, Budde K. Everolimus for angiomyolipoma associated
711 with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a
712 multicentre, randomised, double-blind, placebo-controlled trial. *Lancet*.
713 2013;381(9869):817–24.
- 714 78. Santulli G, Totary-Jain H. Tailoring mTOR-based therapy: molecular evidence and
715 clinical challenges. *Pharmacogenomics*. 2013;14(12):1517–26.
- 716 79. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback
717 activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene*.
718 2007;26(13):1932–40.

- 719 80. Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, Egia A, Sasaki
720 AT, THOMAS G, Kozma SC, Papa A, Nardella C, Cantley LC, Baselga J, Pandolfi PP.
721 Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent
722 feedback loop in human cancer. *J Clin Invest*. 2008;118(9):3065–74.
- 723 81. Sun S-Y, Rosenberg LM, Wang X, Zhou Z, Yue P, Fu H, Khuri FR. Activation of Akt and
724 eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin
725 inhibition. *Cancer Res*. 2005;65(16):7052–8.
- 726 82. O'Reilly KE, Rojo F, She Q-B, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin
727 DJ, Ludwig DL, Baselga J, Rosen N. mTOR inhibition induces upstream receptor
728 tyrosine kinase signaling and activates Akt. *Cancer Res*. 2006;66(3):1500–8.
- 729 83. Choo AY, Yoon S-O, Kim SG, Roux PP, Blenis J. Rapamycin differentially inhibits S6Ks
730 and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proc Natl
731 Acad Sci U S A*. 2008;105(45):17414–9.
- 732 84. Kang SA, Pacold ME, Cervantes CL, Lim D, Lou HJ, Ottina K, Gray NS, Turk BE, Yaffe
733 MB, Sabatini DM. mTORC1 phosphorylation sites encode their sensitivity to starvation
734 and rapamycin. *Science*. 2013;341(6144):1236566–6.
- 735 85. Ruggero D, Montanaro L, Ma L, Xu W, Londei P, Cordon-Cardo C, Pandolfi PP. The
736 translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in
737 lymphomagenesis. *Nat Med*. 2004;10(5):484–6.
- 738 86. Dowling RJO, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X,
739 Larsson O, Selvaraj A, Liu Y, Kozma SC, THOMAS G, Sonenberg N. mTORC1-
740 mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science*.
741 2010;328(5982):1172–6.
- 742 87. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, Shokat KM. Active-
743 Site Inhibitors of mTOR Target Rapamycin-Resistant Outputs of mTORC1 and
744 mTORC2. *PLoS Biol*. 2009;7(2):e1000038.
- 745 88. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T,
746 Sabatini DM, Gray NS. An ATP-competitive Mammalian Target of Rapamycin Inhibitor
747 Reveals Rapamycin-resistant Functions of mTORC1. *J Biol Chem*. 2009;284(12):8023–
748 32.
- 749 89. Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J, Su B.
750 SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation
751 and substrate specificity. *Cell*. 2006;127(1):125–37.
- 752 90. Neri LM, Cani A, Martelli AM, Simioni C, Junghanss C, Tabellini G, Ricci F, Tazzari PL,
753 Pagliaro P, McCubrey JA, Capitani S. Targeting the PI3K/Akt/mTOR signaling pathway
754 in B-precursor acute lymphoblastic leukemia and its therapeutic potential. *Leukemia*.
755 2014;28(4):739–48.
- 756 91. Petrich AM, Leshchenko V, Kuo PY, Xia B, Thirukonda VK, Ulahannan N, Gordon S,
757 Fazzari MJ, Ye BH, Sparano JA, Parekh S. Akt Inhibitors MK-2206 and Nelfinavir
758 Overcome mTOR Inhibitor Resistance in Diffuse Large B-cell Lymphoma. *Clin Cancer*

- 759 Res. 2012;18(9):2534–44.
- 760 92. García-Martínez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, Alessi
761 DR. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR).
762 *Biochem J.* 2009;421(1):29–42.
- 763 93. Pike KG, Malagu K, Hummersone MG, Menear KA, Duggan HME, Gomez S, Martin
764 NMB, Ruston L, Pass SL, Pass M. Optimization of potent and selective dual mTORC1
765 and mTORC2 inhibitors: the discovery of AZD8055 and AZD2014. *Bioorg Med Chem*
766 *Lett.* 2013;23(5):1212–6.
- 767 94. Jessen K, Wang S, Kessler L, Guo X, Kucharski J, Staunton J, Lan L, Elia M, Stewart J,
768 Brown J, Li L, Chan K, Martin M, Ren P, Rommel C, Liu Y. Abstract B148: INK128 is a
769 potent and selective TORC1/2 inhibitor with broad oral antitumor activity. *Mol Cancer*
770 *Ther.* 2009;8(Supplement 1):B148–8.
- 771 95. Mortensen DS, Fultz KE, Xu S, Xu W, Packard G, Khambatta G, Gamez JC, Leisten J,
772 Zhao J, Apuy J, Ghoreishi K, Hickman M, Narla RK, Bissonette R, Richardson S, Peng
773 SX, Perrin-Ninkovic S, Tran T, Shi T, Yang WQ, Tong Z, Cathers BE, Moghaddam MF,
774 Canan SS, Worland P, Sankar S, Raymon HK. CC-223, a Potent and Selective Inhibitor
775 of mTOR Kinase: In Vitro and In Vivo Characterization. *Mol Cancer Ther.*
776 2015;14(6):1295–305.
- 777 96. Hsieh AC, Costa M, Zollo O, Davis C, Feldman ME, Testa JR, Meyuhos O, Shokat KM,
778 Ruggero D. Genetic Dissection of the Oncogenic mTOR Pathway Reveals Druggable
779 Addiction to Translational Control via 4EBP-eIF4E. *Cancer Cell.* 2010;17(3):249–61.
- 780 97. Ono A, Oike R, Okuhashi Y, Takahashi Y, Itoh M, Nara N, Tohda S. Comparative
781 effects of PP242 and rapamycin on mTOR signalling and NOTCH signalling in leukemia
782 cells. *Anticancer Res.* 2013;33(3):809–13.
- 783 98. Mallya S, Fitch BA, Lee JS, So L, Janes MR, Fruman DA. Resistance to mTOR kinase
784 inhibitors in lymphoma cells lacking 4EBP1. Sobol RW, editor. *PLoS ONE.*
785 2014;9(2):e88865.
- 786 99. Janes MR, Limon JJ, So L, Chen J, Lim RJ, Chavez MA, Vu C, Lilly MB, Mallya S, Ong
787 ST, Konopleva M, Martin MB, Ren P, Liu Y, Rommel C, Fruman DA. Effective and
788 selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. *Nat Med.*
789 2010;16(2):205–13.
- 790 100. Gupta M, Hendrickson AEW, Yun SS, Han JJ, Schneider PA, Koh BD, Stenson MJ,
791 Wellik LE, Shing JC, Peterson KL, Flatten KS, Hess AD, Smith BD, Karp JE, Barr S,
792 Witzig TE, Kaufmann SH. Dual mTORC1/mTORC2 inhibition diminishes Akt activation
793 and induces Puma-dependent apoptosis in lymphoid malignancies. *Blood.*
794 2012;119(2):476–87.
- 795 101. Simioni C, Cani A, Martelli AM, Zauli G, Tabellini G, McCubrey J, Capitani S, Neri LM.
796 Activity of the novel mTOR inhibitor Torin-2 in B-precursor acute lymphoblastic
797 leukemia and its therapeutic potential to prevent Akt reactivation. *Oncotarget.*
798 2014;5(20):10034–47.

- 799 102. Janes MR, Vu C, Mallya S, Shieh MP, Limon JJ, Li L-S, Jessen KA, Martin MB, Ren P,
800 Lilly MB, Sender LS, Liu Y, Rommel C, Fruman DA. Efficacy of the investigational
801 mTOR kinase inhibitor MLN0128/INK128 in models of B-cell acute lymphoblastic
802 leukemia. *Leukemia*. 2013;27(3):586–94.
- 803 103. Beagle BR, Nguyen DM, Mallya S, Tang SS, Lu M, Zeng Z, Konopleva M, Vo T-T,
804 Fruman DA. mTOR kinase inhibitors synergize with histone deacetylase inhibitors to kill
805 B-cell acute lymphoblastic leukemia cells. *Oncotarget*. 2015;6(4):2088–100.
- 806 104. Lee JS, Tang SS, Ortiz V, Vo T-T, Fruman DA. MCL-1-independent mechanisms of
807 synergy between dual PI3K/mTOR and BCL-2 inhibition in diffuse large B cell
808 lymphoma. *Oncotarget*. 2015;6(34):35202–17.
- 809 105. Basu B, Dean E, Puglisi M, Greystoke A, Ong M, Burke W, Cavallin M, Bigley G,
810 Womack C, Harrington EA, Green S, Oelmann E, de Bono JS, Ranson M, Banerji U.
811 First-in-Human Pharmacokinetic and Pharmacodynamic Study of the Dual m-TORC 1/2
812 Inhibitor AZD2014. *Clin Cancer Res*. 2015;21(15):3412–9.
- 813 106. Bendell JC, Kelley RK, Shih KC, Grabowsky JA, Bergsland E, Jones S, Martin T,
814 Infante JR, Mischel PS, Matsutani T, Xu S, Wong L, Liu Y, Wu X, Mortensen DS,
815 Chopra R, Hege K, Munster PN. A phase I dose-escalation study to assess safety,
816 tolerability, pharmacokinetics, and preliminary efficacy of the dual mTORC1/mTORC2
817 kinase inhibitor CC-223 in patients with advanced solid tumors or multiple myeloma.
818 *Cancer*. 2015;121(19):3481–90.
- 819 107. Infante JR, Taberero J, Cervantes A, Jalal S. Abstract C252: A phase 1, dose-
820 escalation study of MLN0128, an investigational oral mammalian target of rapamycin
821 complex 1/2 (mTORC1/2) catalytic inhibitor, in patients (pts) with advanced non-
822 hematologic malignancies. *Mol Cancer Ther*. 2013;12:C252.
- 823 108. Bertacchini J, Guida M, Accordi B, Mediani L, Martelli AM, Barozzi P, Petricoin E, Liotta
824 L, Milani G, Giordan M, Luppi M, Forghieri F, De Pol A, Cocco L, Basso G, Marmiroli S.
825 Feedbacks and adaptive capabilities of the PI3K/Akt/mTOR axis in acute myeloid
826 leukemia revealed by pathway selective inhibition and phosphoproteome analysis.
827 *Leukemia*. 2014;28(11):2197–205.
- 828 109. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M,
829 Moskatel E, Baselga J, Guichard S, Rosen N. mTOR kinase inhibition causes feedback-
830 dependent biphasic regulation of AKT signaling. *Cancer Discov*. 2011;1(3):248–59.
- 831 110. García-García C, Ibrahim YH, Serra V, Calvo MT, Guzmán M, Grueso J, Aura C, Pérez
832 J, Jessen K, Liu Y, Rommel C, Taberero J, Baselga J, Scaltriti M. Dual mTORC1/2
833 and HER2 blockade results in antitumor activity in preclinical models of breast cancer
834 resistant to anti-HER2 therapy. *Clin Cancer Res*. 2012;18(9):2603–12.
- 835 111. Alain T, Sonenberg N, Topisirovic I. mTOR inhibitor efficacy is determined by the
836 eIF4E/4E-BP ratio. *Oncotarget*. 2012;3(12):1491–2.
- 837 112. Schatz JH, Oricchio E, Wolfe AL, Jiang M, Linkov I, Maragulia J, Shi W, Zhang Z,
838 Rajasekhar VK, Pagano NC, Porco JA, Teruya-Feldstein J, Rosen N, Zelenetz AD,
839 Pelletier J, Wendel H-G. Targeting cap-dependent translation blocks converging

- 840 survival signals by AKT and PIM kinases in lymphoma. *J Exp Med.* 2011;208(9):1799–
841 807.
- 842 113. Harada M, Benito J, Yamamoto S, Kaur S, Arslan D, Ramirez S, Jacamo R, Plataniias
843 L, Matsushita H, Fujimura T, Kazuno S, Kojima K, Tabe Y, Konopleva M. The novel
844 combination of dual mTOR inhibitor AZD2014 and pan-PIM inhibitor AZD1208 inhibits
845 growth in acute myeloid leukemia via HSF pathway suppression. *Oncotarget.*
846 2015;6(35):37930–47.
- 847 114. Aparicio CB, Renner O, Gomez-Casero E. Abstract A275: Co-targeting PIM and
848 PI3K/mTOR pathways with a single molecule: Novel orally available combined
849 PIM/PI3K and PIM/PI3K/mTOR kinase inhibitors. *Mol Cancer Ther.* 2013;12(11
850 Suppl):A275.
- 851 115. Marzec M, Liu X, Wysocka M, Rook AH, Odum N, Wasik MA. Simultaneous inhibition of
852 mTOR-containing complex 1 (mTORC1) and MNK induces apoptosis of cutaneous T-
853 cell lymphoma (CTCL) cells. *PLoS ONE.* 2011;6(9):e24849.
- 854 116. André F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S,
855 Arena F, Isaacs C, Yap Y-S, Papai Z, Lang I, Armstrong A, Lerzo G, White M, Shen K,
856 Litton J, Chen D, Zhang Y, Ali S, Taran T, Gianni L. Everolimus for women with
857 trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a
858 randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol.*
859 2014;15(6):580–91.
- 860 117. Shimizu T, Tolcher AW, Papadopoulos KP, Beeram M, Rasco DW, Smith LS, Gunn S,
861 Smetzer L, Mays TA, Kaiser B, Wick MJ, Alvarez C, Cavazos A, Mangold GL, Patnaik
862 A. The clinical effect of the dual-targeting strategy involving PI3K/AKT/mTOR and
863 RAS/MEK/ERK pathways in patients with advanced cancer. *Clin Cancer Res.*
864 2012;18(8):2316–25.
- 865 118. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis
866 J, Greenberg ME. Akt Promotes Cell Survival by Phosphorylating and Inhibiting a
867 Forkhead Transcription Factor. *Cell.* 1999;96(6):857–68.
- 868 119. Dijkers PF, Medema RH, Lammers J-WJ, Koenderman L, Coffey PJ. Expression of the
869 pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor
870 FKHR-L1. *Curr Biol.* 2000;10(19):1201–4.
- 871 120. Sarosiek KA, Chi X, Bachman JA, Sims JJ, Montero J, Patel L, Flanagan A, Andrews
872 DW, Sorger P, Letai A. BID Preferentially Activates BAK while BIM Preferentially
873 Activates BAX, Affecting Chemotherapy Response. *Molecular Cell.* 2013;51(6):751–65.
- 874 121. Coloff JL, Macintyre AN, Nichols AG, Liu T, Gallo CA, Plas DR, Rathmell JC. Akt-
875 Dependent Glucose Metabolism Promotes Mcl-1 Synthesis to Maintain Cell Survival
876 and Resistance to Bcl-2 Inhibition. *Cancer Res.* 2011;71(15):5204–13.
- 877 122. Ackler S, Xiao Y, Mitten MJ, Foster K, Oleksijew A, Refici M, Schlessinger S, Wang B,
878 Chemburkar SR, Bauch J, Tse C, Frost DJ, Fesik SW, Rosenberg SH, Elmore SW,
879 Shoemaker AR. ABT-263 and rapamycin act cooperatively to kill lymphoma cells in vitro
880 and in vivo. *Mol Cancer Ther.* 2008;7(10):3265–74.

- 881 123. Rahmani M, Aust MM, Attkisson E, Williams DC, Ferreira-Gonzalez A, Grant S. Dual
882 inhibition of Bcl-2 and Bcl-xL strikingly enhances PI3K inhibition-induced apoptosis in
883 human myeloid leukemia cells through a GSK3- and Bim-dependent mechanism.
884 *Cancer Res.* 2013;73(4):1340–51.
- 885 124. Iacovelli S, Ricciardi MR, Allegretti M, Mirabilii S, Licchetta R, Bergamo P, Rinaldo C,
886 Zeuner A, Foà R, Milella M, McCubrey JA, Martelli AM, Tafuri A. Co-targeting of Bcl-2
887 and mTOR pathway triggers synergistic apoptosis in BH3 mimetics resistant acute
888 lymphoblastic leukemia. *Oncotarget.* 2015;6(31):32089–103.
- 889 125. Rudin CM, Hann CL, Garon EB, Ribeiro de Oliveira M, Bonomi PD, Camidge DR, Chu
890 Q, Giaccone G, Khaira D, Ramalingam SS, Ranson MR, Dive C, McKeegan EM, Chyla
891 BJ, Dowell BL, Chakravarty A, Nolan CE, Rudersdorf N, Busman TA, Mabry MH,
892 Krivoshik AP, Humerickhouse RA, Shapiro GI, Gandhi L. Phase II Study of Single-
893 Agent Navitoclax (ABT-263) and Biomarker Correlates in Patients with Relapsed Small
894 Cell Lung Cancer. *Clin Cancer Res.* 2012;18(11):3163–9.
- 895 126. Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding
896 H, Enschede SH, Fairbrother WJ, Huang DCS, Hymowitz SG, Jin S, Khaw SL, Kovar
897 PJ, Lam LT, Lee J, Maecker HL, Marsh KC, Mason KD, Mitten MJ, Nimmer PM,
898 Oleksijew A, Park CH, Park C-M, Phillips DC, Roberts AW, Sampath D, Seymour JF,
899 Smith ML, Sullivan GM, Tahir SK, Tse C, Wendt MD, Xiao Y, Xue JC, Zhang H,
900 Humerickhouse RA, Rosenberg SH, Elmore SW. ABT-199, a potent and selective BCL-
901 2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med.*
902 2013;19(2):202–8.
- 903 127. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, Kipps TJ,
904 Anderson M-A, Brown JR, Gressick L, Wong S, Dunbar M, Zhu M, Desai MB, Cerri E,
905 Enschede SH, Humerickhouse RA, Wierda WG, Seymour JF. Targeting BCL2 with
906 Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med.*
907 2015;:151206090218007.
- 908 128. Choudhary GS, Al-harbi S, Mazumder S, Hill BT, Smith MR, Bodo J, Hsi ED, Almasan
909 A. MCL-1 and BCL-xL-dependent resistance to the BCL-2 inhibitor ABT-199 can be
910 overcome by preventing PI3K/AKT/mTOR activation in lymphoid malignancies. *Cell*
911 *Death Dis.* 2015;6(1):e1593.
- 912 129. Fiskus W, Verstovsek S, Manshour T, Smith JE, Peth K, Abhyankar S, McGuirk J,
913 Bhalla KN. Dual PI3K/AKT/mTOR inhibitor BEZ235 synergistically enhances the activity
914 of JAK2 inhibitor against cultured and primary human myeloproliferative neoplasm cells.
915 *Mol Cancer Ther.* 2013;12(5):577–88.
- 916 130. Bogani C, Bartalucci N, Martinelli S, Tozzi L, Guglielmelli P, Bosi A, Vannucchi AM,
917 Associazione Italiana per la Ricerca sul Cancro AGIMM Gruppo Italiano Malattie
918 Mieloproliferative. mTOR inhibitors alone and in combination with JAK2 inhibitors
919 effectively inhibit cells of myeloproliferative neoplasms. Tse W, editor. *PLoS ONE.*
920 2013;8(1):e54826.
- 921 131. Mathews Griner LA, Guha R, Shinn P, Young RM, Keller JM, Liu D, Goldlust IS, Yasgar
922 A, McKnight C, Boxer MB, Duveau DY, Jiang J-K, Michael S, Mierzwa T, Huang W,
923 Walsh MJ, Mott BT, Patel P, Leister W, Maloney DJ, Leclair CA, Rai G, Jadhav A,

- 924 Peysner BD, Austin CP, Martin SE, Simeonov A, Ferrer M, Staudt LM, Thomas CJ. High-
925 throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill
926 activated B-cell-like diffuse large B-cell lymphoma cells. *Proc Natl Acad Sci U S A*.
927 2014;111(6):2349–54.
- 928 132. Ezell SA, Mayo M, Bihani T, Tepsuporn S, Wang S, Passino M, Grosskurth SE, Collins
929 M, Parmentier J, Reimer C, Byth KF. Synergistic induction of apoptosis by combination
930 of BTK and dual mTORC1/2 inhibitors in diffuse large B cell lymphoma. *Oncotarget*.
931 2014;5(13):4990–5001.
- 932 133. Huang S, Yang ZJ, Yu C, Sinicrope FA. Inhibition of mTOR kinase by AZD8055 can
933 antagonize chemotherapy-induced cell death through autophagy induction and down-
934 regulation of p62/sequestosome 1. *J Biol Chem*. 2011;286(46):40002–12.
- 935 134. White E, DiPaola RS. The double-edged sword of autophagy modulation in cancer. *Clin
936 Cancer Res*. 2009;15(17):5308–16.
- 937 135. Zhuang S. Regulation of STAT signaling by acetylation. *Cell Signal*. 2013;25(9):1924–
938 31.
- 939 136. Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, Rocha K, Kumaraswamy S,
940 Boyapalle S, Atadja P, Seto E, Bhalla K. Inhibition of histone deacetylase 6 acetylates
941 and disrupts the chaperone function of heat shock protein 90: a novel basis for
942 antileukemia activity of histone deacetylase inhibitors. *J Biol Chem*.
943 2005;280(29):26729–34.
- 944 137. Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimochi K, Fukamizu A. Acetylation of
945 Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. *Proc Natl Acad
946 Sci U S A*. 2005;102(32):11278–83.
- 947 138. van der Heide LP, Smidt MP. Regulation of FoxO activity by CBP/p300-mediated
948 acetylation. *Trends Biochem Sci*. 2005;30(2):81–6.
- 949 139. Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL,
950 Holmfeldt L, Collins-Underwood JR, Ma J, Buetow KH, Pui C-H, Baker SD, Brindle PK,
951 Downing JR. CREBBP mutations in relapsed acute lymphoblastic leukaemia. *Nature*.
952 2011; 471(7337):235–9.
- 953 140. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper
954 LH, Lerach S, Tang H, Ma J, Rossi D, Chadburn A, Murty VV, Mullighan CG, Gaidano
955 G, Rabadan R, Brindle PK, Dalla-Favera R. Inactivating mutations of acetyltransferase
956 genes in B-cell lymphoma. *Nature*. 2011;471(7337):189–95.
- 957 141. Bereshchenko OR, Gu W, Dalla-Favera R. Acetylation inactivates the transcriptional
958 repressor BCL6. *Nat Genet*. 2002;32(4):606–13.
- 959 142. Basso K, Dalla-Favera R. Germinal centres and B cell lymphomagenesis. *Nat Rev
960 Immunol*. 2015;15(3):172–84.
- 961 143. Duan H, Heckman CA, Boxer LM. Histone deacetylase inhibitors down-regulate bcl-2
962 expression and induce apoptosis in t(14;18) lymphomas. *Mol Cell Biol*.

- 963 2005;25(5):1608–19.
- 964 144. Zhao Y, Tan J, Zhuang L, Jiang X, Liu ET, Yu Q. Inhibitors of histone deacetylases
965 target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic
966 protein Bim. *Proc Natl Acad Sci U S A.* 2005;102(44):16090–5.
- 967 145. Sakajiri S, Kumagai T, Kawamata N, Saitoh T, Said JW, Koeffler HP. Histone
968 deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell
969 lines. *Exp Hematol.* 2005;33(1):53–61.
- 970 146. Rahmani M, Reese E, Dai Y, Bauer C, Payne SG, Dent P, Spiegel S, Grant S.
971 Coadministration of histone deacetylase inhibitors and perifosine synergistically induces
972 apoptosis in human leukemia cells through Akt and ERK1/2 inactivation and the
973 generation of ceramide and reactive oxygen species. *Cancer Res.* 2005;65(6):2422–32.
- 974 147. Insinga A, Monestiroli S, Ronzoni S, Gelmetti V, Marchesi F, Viale A, Altucci L, Nervi C,
975 Minucci S, Pelicci PG. Inhibitors of histone deacetylases induce tumor-selective
976 apoptosis through activation of the death receptor pathway. *Nat Med.* 2005;11(1):71–6.
- 977 148. Heider U, Kaiser M, Sterz J, Zavrski I, Jakob C, Fleissner C, Eucker J, Possinger K,
978 Sezer O. Histone deacetylase inhibitors reduce VEGF production and induce growth
979 suppression and apoptosis in human mantle cell lymphoma. *Eur J Haematol.*
980 2006;76(1):42–50.
- 981 149. Yazbeck VY, Buglio D, Georgakis GV, Li Y, Iwado E, Romaguera JE, Kondo S, Younes
982 A. Temsirolimus downregulates p21 without altering cyclin D1 expression and induces
983 autophagy and synergizes with vorinostat in mantle cell lymphoma. *Exp Hematol.*
984 2008;36(4):443–50.
- 985 150. Haritunians T, Mori A, O'Kelly J, Luong QT, Giles FJ, Koeffler HP. Antiproliferative
986 activity of RAD001 (everolimus) as a single agent and combined with other agents in
987 mantle cell lymphoma. *Leukemia.* 2007;21(2):333–9.
- 988 151. Rahmani M, Aust MM, Benson EC, Wallace L, Friedberg J, Grant S. PI3K/mTOR
989 inhibition markedly potentiates HDAC inhibitor activity in NHL cells through BIM- and
990 MCL-1-dependent mechanisms in vitro and in vivo. *Clin Cancer Res.*
991 2014;20(18):4849–60.
- 992 152. Oki Y, Buglio D, Fanale M, Fayad L, Copeland A, Romaguera J, Kwak LW, Pro B, de
993 Castro Faria S, Neelapu S, Fowler N, Hagemester F, Zhang J, Zhou S, Feng L, Younes
994 A. Phase I study of panobinostat plus everolimus in patients with relapsed or refractory
995 lymphoma. *Clin Cancer Res.* 2013;19(24):6882–90.
- 996 153. Almond JB, Cohen GM. The proteasome: a novel target for cancer chemotherapy.
997 *Leukemia.* 2002;16(4):433–43.
- 998 154. Pérez-Galán P, Roué G, Villamor N, Montserrat E, Campo E, Colomer D. The
999 proteasome inhibitor bortezomib induces apoptosis in mantle-cell lymphoma through
1000 generation of ROS and Noxa activation independent of p53 status. *Blood.*
1001 2006;107(1):257–64.

- 1002 155. Strauss SJ, Higginbottom K, Jülicher S, Maharaj L, Allen P, Schenkein D, Lister TA, Joel
1003 SP. The proteasome inhibitor bortezomib acts independently of p53 and induces cell
1004 death via apoptosis and mitotic catastrophe in B-cell lymphoma cell lines. *Cancer Res.*
1005 2007;67(6):2783–90.
- 1006 156. O'Connor OA, Wright J, Moskowitz C, Muzzy J, MacGregor-Cortelli B, Stubblefield M,
1007 Straus D, Portlock C, Hamlin P, Choi E, Dumetrescu O, Esseltine D, Trehu E, Adams J,
1008 Schenkein D, Zelenetz AD. Phase II clinical experience with the novel proteasome
1009 inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell
1010 lymphoma. *J Clin Oncol.* 2005;23(4):676–84.
- 1011 157. Goy A, Younes A, McLaughlin P, Pro B, Romaguera JE, Hagemester F, Fayad L, Dang
1012 NH, Samaniego F, Wang M, Broglio K, Samuels B, Gilles F, Sarris AH, Hart S, Trehu E,
1013 Schenkein D, Cabanillas F, Rodriguez AM. Phase II study of proteasome inhibitor
1014 bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. *J Clin Oncol.*
1015 2005;23(4):667–75.
- 1016 158. Fisher RI, Bernstein SH, Kahl BS, Djulbegovic B, Robertson MJ, de Vos S, Epner E,
1017 Krishnan A, Leonard JP, Lonial S, Stadtmauer EA, O'Connor OA, Shi H, Boral AL, Goy
1018 A. Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle
1019 cell lymphoma. *J Clin Oncol.* 2006;24(30):4867–74.
- 1020 159. Ruan J, Martin P, Furman RR, Lee SM, Cheung K, Vose JM, Lacasce A, Morrison J,
1021 Elstrom R, Ely S, Chadburn A, Cesarman E, Coleman M, Leonard JP. Bortezomib plus
1022 CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell
1023 lymphoma. *J Clin Oncol.* 2011;29(6):690–7.
- 1024 160. Kane RC, Dagher R, Farrell A, Ko C-W, Sridhara R, Justice R, Pazdur R. Bortezomib
1025 for the treatment of mantle cell lymphoma. *Clin Cancer Res.* 2007;13(18 Pt 1):5291–4.
- 1026 161. Crawford LJ, Walker B, Irvine AE. Proteasome inhibitors in cancer therapy. *J Cell*
1027 *Commun Signal.* 2011;5(2):101–10.
- 1028 162. McConkey DJ, Zhu K. Mechanisms of proteasome inhibitor action and resistance in
1029 cancer. *Drug Resist Updat.* 2008;11(4-5):164–79.
- 1030 163. Paoluzzi L, O'Connor OA. Mechanistic rationale and clinical evidence for the efficacy of
1031 proteasome inhibitors against indolent and mantle cell lymphomas. *BioDrugs.*
1032 2006;20(1):13–23.
- 1033 164. Pham LV, Tamayo AT, Yoshimura LC. Inhibition of constitutive NF- κ B activation in
1034 mantle cell lymphoma B cells leads to induction of cell cycle arrest and apoptosis. *J*
1035 *Immunol.* 2003;171(1):88–95.
- 1036 165. Milhollen MA, Traore T, Adams-Duffy J, Thomas MP, Berger AJ, Dang L, Dick LR,
1037 Garnsey JJ, Koenig E, Langston SP, Manfredi M, Narayanan U, Rolfe M, Staudt LM,
1038 Soucy TA, Yu J, Zhang J, Bolen JB, Smith PG. MLN4924, a NEDD8-activating enzyme
1039 inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of
1040 NF- κ B-dependent lymphoma. *Blood.* 2010;116(9):1515–23.
- 1041 166. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, Wright G, Grant N, Shovlin M, Jaffe

- 1042 ES, Janik JE, Staudt LM, Wilson WH. Differential efficacy of bortezomib plus
1043 chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. *Blood*.
1044 2009;113(24):6069–76.
- 1045 167. Di Bella N, Taetle R, Kolibaba K, Boyd T, Raju R, Barrera D, Cochran EW, Dien PY,
1046 Lyons R, Schlegel PJ, Vukelja SJ, Boston J, Boehm KA, Wang Y, Asmar L. Results of a
1047 phase 2 study of bortezomib in patients with relapsed or refractory indolent lymphoma.
1048 *Blood*. 2010;115(3):475–80.
- 1049 168. Ribrag V, Tilly H, Casasnovas O, Bosly A, Bouabdallah R, Delarue R, Boue F, Bron D,
1050 Feugier P, Haioun C, Offner F, Coiffier B. Efficacy and toxicity of two schedules of
1051 bortezomib in patients with recurrent or refractory follicular lymphoma: a randomised
1052 phase II trial from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). *Eur J*
1053 *Cancer*. 2013;49(4):904–10.
- 1054 169. O'Connor OA, Portlock C, Moskowitz C, Hamlin P, Straus D, Gerecitano J, Gonen M,
1055 Dumitrescu O, Sarasohn D, Butos J, Neylon E, Mac-Gregor Cortelli B, Blumel S, Evens
1056 AM, Zelenetz AD, Wright J, Cooper B, Winter J, Vose J. Time to treatment response in
1057 patients with follicular lymphoma treated with bortezomib is longer compared with other
1058 histologic subtypes. *Clin Cancer Res*. 2010;16(2):719–26. 164. Uziel O, Cohen O,
1059 Beery E, Nordenberg J, Lahav M. The effect of Bortezomib and Rapamycin on
1060 Telomerase Activity in Mantle Cell Lymphoma. *Transl Oncol*. 2014 Dec;7(6):741–51.
- 1061 170. Uziel O, Cohen O, Beery E, Nordenberg J, Lahav M. The effect of Bortezomib and
1062 Rapamycin on Telomerase Activity in Mantle Cell Lymphoma. *Transl Oncol*.
1063 2014;7(6):741–51.
- 1064 171. Kim JE, Yoon DH, Jang G, Lee DH, Kim S, Park C-S, Huh J, Kim WS, Park J, Lee JH,
1065 Lee SI, Suh C. A phase I/II study of bortezomib plus CHOP every 2 weeks (CHOP-14)
1066 in patients with advanced-stage diffuse large B-cell lymphomas. *Korean J Hematol*.
1067 2012 Mar;47(1):53–9.
- 1068 172. Mounier N, Ribrag V, Haioun C, Salles G, Golfier J, Ertault M, Ferme C, Briere J, Brice
1069 P, De Kerviler E, Gisselbrecht C. Abstract 8010: Efficacy and toxicity of two schedules
1070 of R-CHOP plus bortezomib in front-line B lymphoma patients: A randomized phase II
1071 trial from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). *J Clin Oncol*.
1072 2007;25(18S).
- 1073 173. Tobinai K, Ogura M, Maruyama D, Uchida T, Uike N, Choi I, Ishizawa K, Itoh K, Ando K,
1074 Taniwaki M, Shimada N, Kobayashi K. Phase I study of the oral mammalian target of
1075 rapamycin inhibitor everolimus (RAD001) in Japanese patients with relapsed or
1076 refractory non-Hodgkin lymphoma. *Int J Hematol*. 2010;92(4):563–70.
- 1077 174. Barnes JA, Jacobsen E, Feng Y, Freedman A, Hochberg EP, LaCasce AS, Armand P,
1078 Joyce R, Sohani AR, Rodig SJ, Neuberger D, Fisher DC, Abramson JS. Everolimus in
1079 combination with rituximab induces complete responses in heavily pretreated diffuse
1080 large B-cell lymphoma. *Haematologica*. 2013;98(4):615–9.

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1083 **Figure Legends**

1084 **Figure 1: mTOR signaling pathway**

1085 mTOR exists in two distinct complexes (mTORC1 and mTORC2) that are regulated separately
1086 and have distinct substrates. Whereas mTORC2 is regulated downstream of PI3K, mTORC1 is
1087 coordinately regulated by growth factor signals, nutrient availability (amino acids), and cellular
1088 energy status (ATP levels). The outputs of their downstream effectors coordinate processes
1089 required for cell growth including survival, inhibition of autophagy, protein translation and cell
1090 cycle progression.

1091

1092 **Figure 2: Mechanisms of aberrant mTOR activation in B-cell malignancies**

1093 Different proteins are amplified or activated (shown in red) in B-cell malignancies that result in
1094 increased mTOR activity. Where as the loss of the tumor suppressor PTEN (shown in blue)
1095 promotes mTOR activation. These mutations negate the normal constraints on mTOR activity
1096 to promote cancer cell proliferation.

1097

1098 **Figure 3: Different effects of rapamycin and TOR-KI on mTOR activity**

1099 Rapamycin (and all rapalogs) only partially inhibits mTOR activity. Rapamycin forms a complex
1100 with FKB12 to inhibits TORC1 activation of S6K activity and only partially reduces effects on
1101 4EBP. S6K normally negatively feeds back to inhibit the activation of PI3K. By suppressing
1102 S6K, rapamycin negates the feedback inhibition resulting in increased PI3K, TORC2 and AKT
1103 activation. Thus, survival signals from AKT highly active and 4EBP is partially active.
1104 Conversely, TOR-KIs suppresses all mTOR survival outputs.

1105

1106 **Figure 4: Combination of targeting BCL-2 and mTOR**

1107 (A) The BCL-2 family of proteins consists of pro- and anti-apoptotic members that interact
1108 antagonistically to determine cell fate. Survival signaling through AKT and mTOR increases the

1109 anti-apoptotic family members and decreases the pro-apoptotic members. (B) By inhibiting
1110 mTOR activity the balance is shifted where anti-apoptotic family members are reduced and pro-
1111 apoptotic members are increased. This reduces the capacity of anti-apoptotic proteins to
1112 sequester the pro-apoptotic proteins. Addition of ABT-199, a BCL-2 inhibitor, further shifts this
1113 balance to release the pro-apoptotic proteins and cause cancer killing.
1114

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

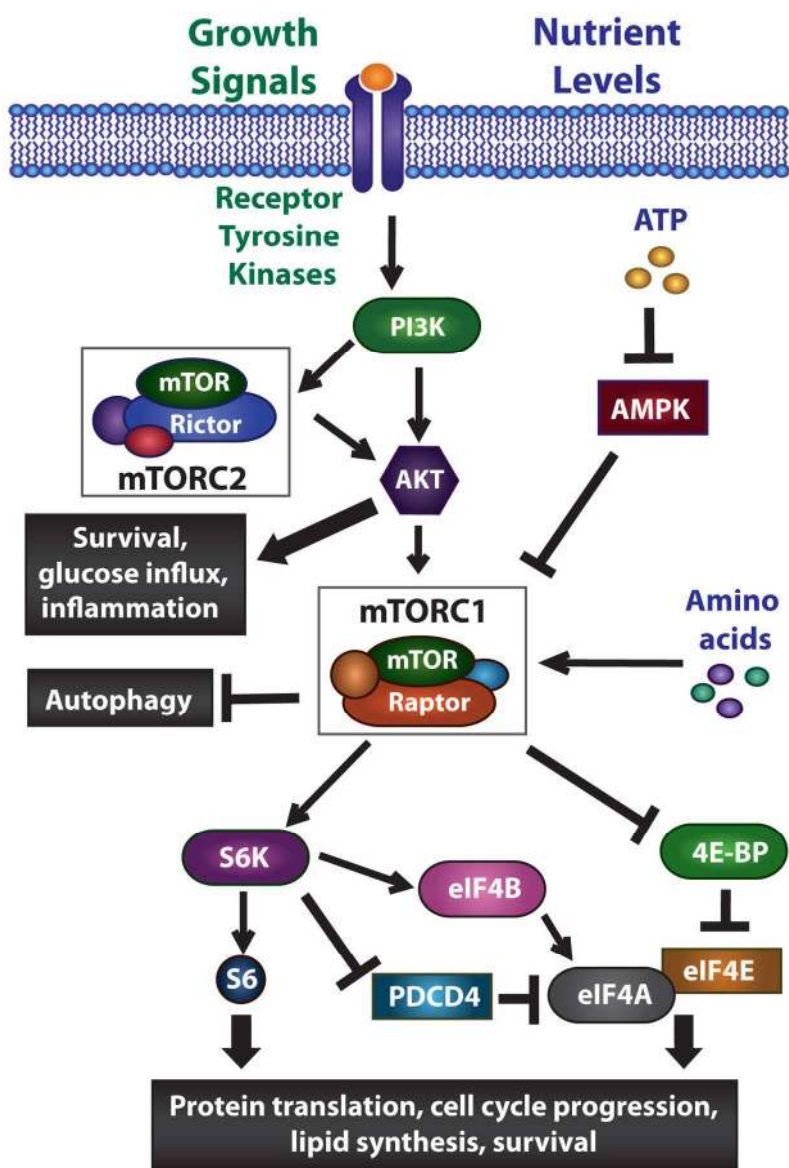


Figure 1
122x171mm (300 x 300 DPI)

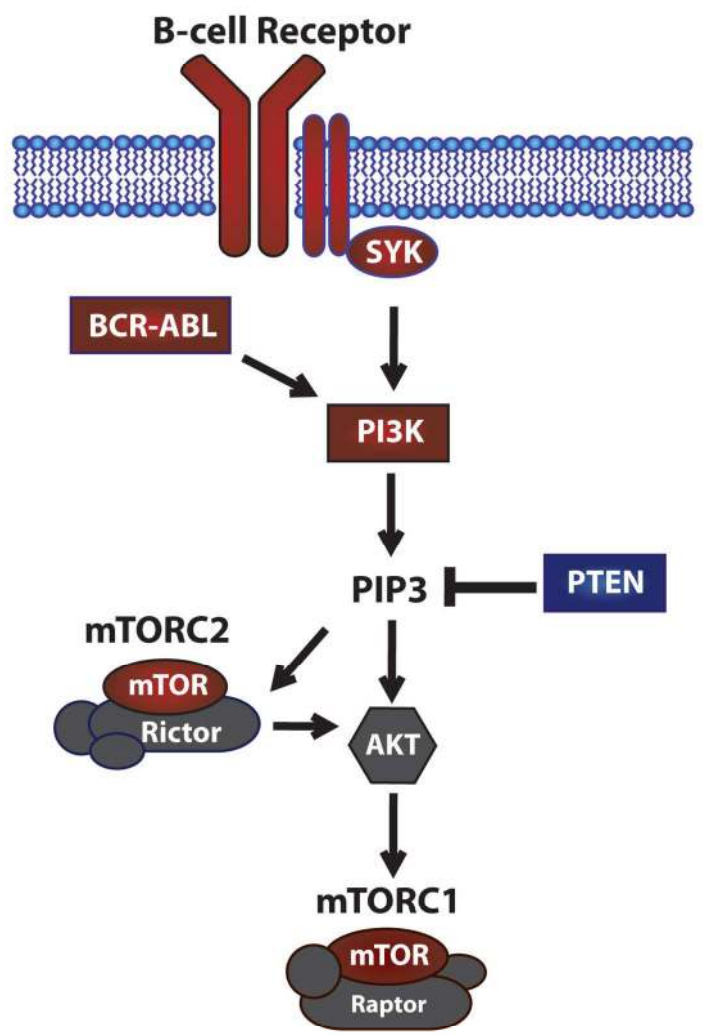


Figure 2
153x225mm (300 x 300 DPI)

British

Pharmacology

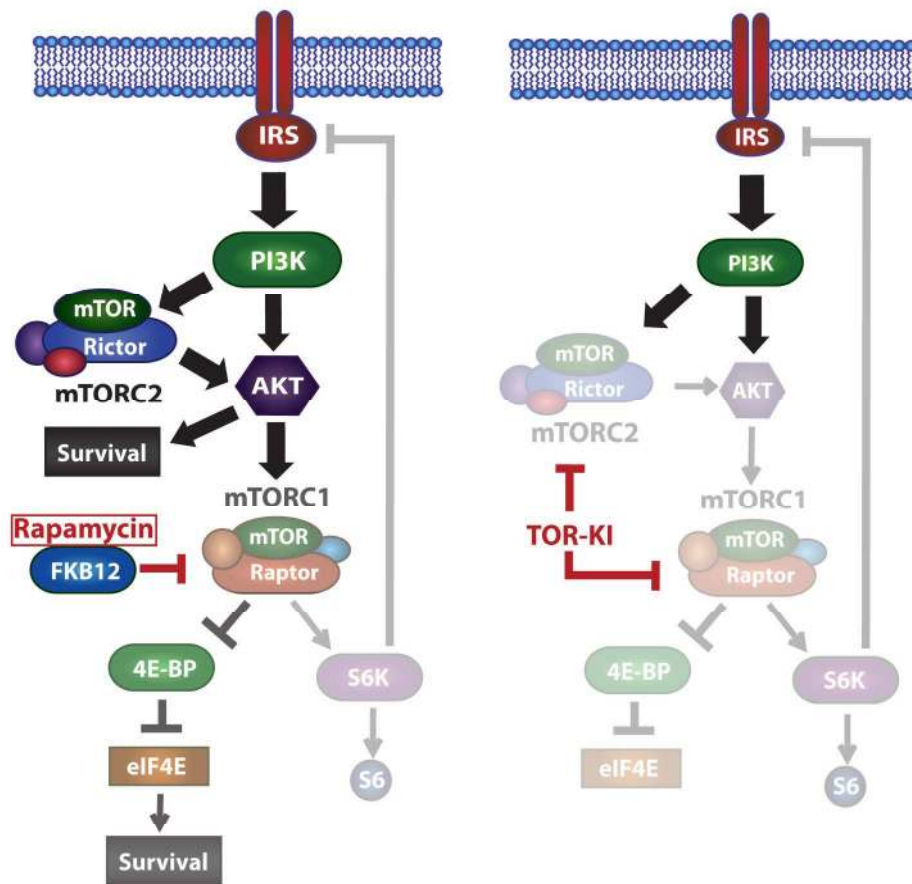


Figure 3
172x164mm (300 x 300 DPI)

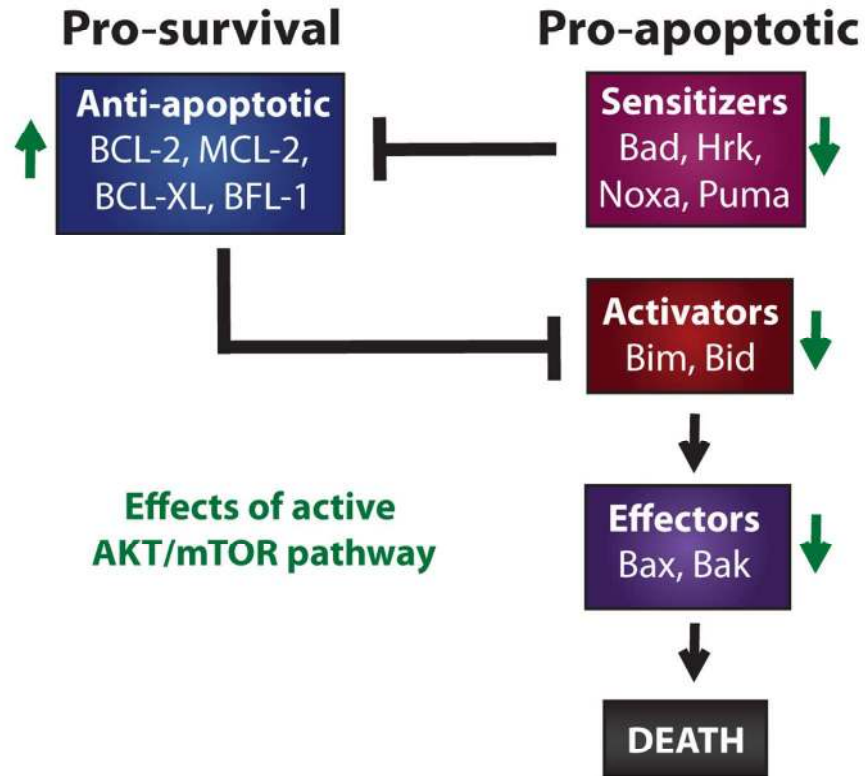


Figure 4A
108x100mm (300 x 300 DPI)

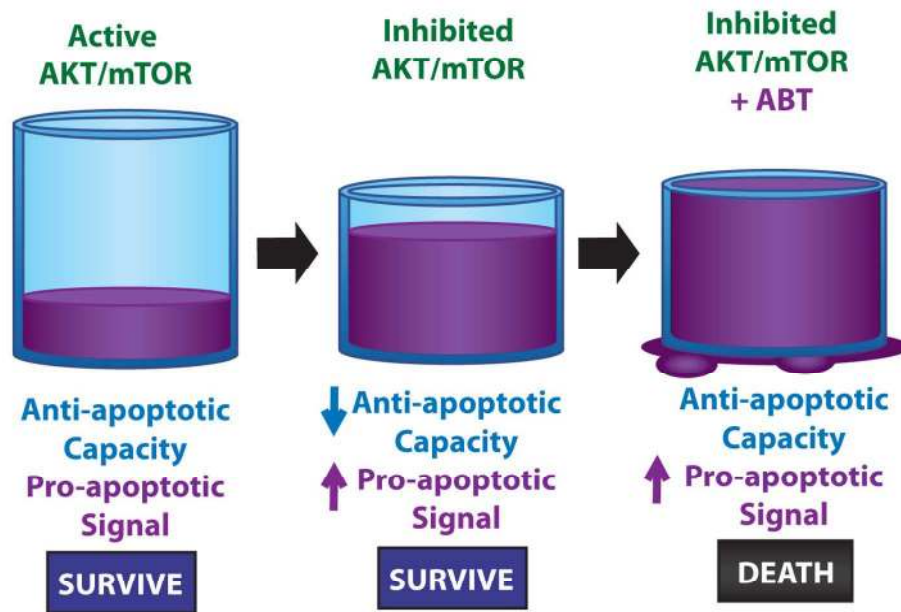


Figure 4B
132x96mm (300 x 300 DPI)