Precision medicines for B-cell malignancies; progress and potential pitfalls.

Martin JS Dyer, Meike Vogler, Jesvin Samuel[1,2], Sandrine Jayne[2], Simon Wagner, Catrin Pritchard[1], Salvador Macip[1]

From the Departments of Cancer Studies and Molecular Medicine and [1]Biochemistry, University of Leicester and [2]MRC Toxicology Unit, Leicester.

Please address correspondence to either:-

Dr Salvador Macip

Sm460@le.ac.uk

Or Professor Martin Dyer

Mjsd1@le.ac.uk

Abstract.

There is now a plethora of new precision medicines for B-cell malignancy including “classical” kinase inhibitors, rationally designed inhibitors of anti-apoptotic proteins and antibody or antibody drug/toxin conjugates with functional properties. Some of these medicines are showing spectacular single agent activity in early phase clinical studies and may reduce or, in combination, even obviate the need for chemotherapy. Nevertheless, significant problems remain if these medicines are to be introduced into routine clinical practice in a rational and affordable manner. Firstly, precision medicines must be carefully matched in a mechanistic fashion with specific subtypes of disease. Whilst sensitivity may be predicted by the detection of key mutations or by expression of target molecules, for therapies that depend on intact intracellular signalling pathways, functional assessment on viable primary malignant cells will be necessary using assays that faithfully mimic *in vivo* conditions. A second and no less important challenge is to define mechanism-based synergistic combinations associated with minimal toxicities rather than simply adding new precision medicines to existing chemotherapeutic regimens. Finally, a closer, open, two-way interaction between academic medicine and the pharmaceutical industry will be necessary to achieve these aims. Implementing such changes would change radically how and where patients with B-cell malignancies are managed.

Introduction.

Despite the cost of introducing a new treatment to the market being in the order of US$1 billion and despite many failing clinically at early stages of development, there are many new, and apparently effective precision medicines being assessed in B-cell malignancy[1]. Some of these are depicted in Table 1; all are showing promising single agent activity in early phase clinical studies. Unlike chronic myeloid leukaemia, where one class of tyrosine kinase inhibitors is sufficient for all cases (due to the presence of the underlying chromosomal translocation t(9;22)(q34;q12)), these agents are very diverse reflecting the different genetic backgrounds and biology of the B-cell malignancies. They include small molecule inhibitors of enzymes (principally kinases), new antibodies constructs (many with enhanced functional activities, including bispecific molecules that can redirect T-cell specificity), antibody-toxin conjugates that finally seem to be delivering clinically their long-observed *in vitro* potential, and a novel and exciting class of molecules that inhibit specific protein-protein interactions to induce apoptosis. The "market" for B-cell malignancies is becoming increasingly "crowded", and from a clinical trials perspective it is becoming increasingly difficult to prioritise new molecules.

In this paper we discuss how to introduce these new agents into routine clinical practice in a rational and affordable way. To date, precision medicines have been largely introduced in an empirical manner, following the precedents established in the last century. The remarkable progress made during the age of empirical medicine in the latter half of the 20th century in terms of leukaemia and lymphoma therapy depended on the concomitant administration of drugs with different modes of action, but with only minimal knowledge of the disease biology. Individual drugs were given at maximally tolerated doses on the premise that more might be better. Empirical principles have been applied in the 21st century to modern medicines. For example, many clinical trials of precision medicines are initiated based on data derived from preclinical testing of *in vitro* adapted cell lines, often assessed in mouse xenograft models, with minimal assessment on primary tumour cells and with no prior individualized patient testing. Many biological agents, and particularly monoclonal antibodies (MAbs) have no maximally tolerated dose (MTD), often resulting in massive doses being administered[2]. (Whether the effects of such large and presumably super-saturating MAb doses are due to specific antigen binding or to the non-specific effects of large doses of immunoglobulin on the

immune system is not clear[3].) To obtain a product licence, new targeted molecules have been simply added to pre-existing chemotherapy regimens, with minimal or no consideration to the underlying biology of disease. Sometimes, this empirical approach has been very successful (for example, the addition of rituximab to chemotherapy for DLBCL and CLL) and sometimes not (for example the use of lumiliximab in CLL (http://www.biogenidec.com/,clinical trial ID NCT00801060) and the development of TRAIL-targeted reagents[4, 5] (clinical trial ID NCT00094848)). Addition of new agents to pre-existing chemotherapy regimens can on occasion have quite unanticipated and unwelcome effects and toxicities and shorten survival [6]. Nevertheless, an empirical strategy has been most widely adopted by industry, since it is usually the quickest way of obtaining financial return on considerable investment. For many malignancies however, results of targeted therapy to date have not been hugely successful, leading some to doubt the merits of this approach[7].

For B-cell malignancies, we suggest a mechanism-based approach, based on functional *in vitro* testing of viable primary malignant B-cells under conditions that closely mimic those *in vivo*, integrating genomic mutational and gene expression data with functional data. This would require more extensive study of individual primary clinical material and much closer patient monitoring than has been used in the past, but ultimately would maximize efficacy and minimize toxicities (both physical and financial). If successful, this approach would have major implications for how new medicines are introduced and assessed and ultimately, how and where oncological medicine is practiced. To achieve these aims however will require close, open and two-way interactions between the pharmaceutical industry and academic haematologists, with the latter being more intimately involved in the design and assessment of early phase clinical trials.

Barriers to the successful introduction of precision medicines.

There are several inter-related practical barriers that hinder the rapid translation of new precision medicines into significant and affordable improvements in clinical outcome.

a) Functional testing of primary patient material under conditions mimicking those *in vivo* is essential to predict drug sensitivity.

The successful introduction of genuinely "personalized" cancer treatments will depend on the ability to predict individual tumour sensitivity not only to single agents but also to combinations of precision medicines. Targeted therapies should be tailored for specific subgroups of patients, using drugs that ideally target either directly or indirectly, the genetic and biochemical abnormalities underlying the different forms of malignancy. Thus, both the introduction and subsequent routine use of precision medicines should be based on a mechanistic basis, assessing the effects of these new agents on primary cells under conditions that mimic those *in vivo*. Such individualized patient testing to predict response in malignancy is by no means a new concept, having been proposed many years ago and pursued subsequently by many groups[8, 9]. However, what is new in the 21st century is both the ability to acquire comprehensive "global" knowledge about abnormalities in the key pathways that "drive" malignancy through the use of genomic and metabolomic strategies, and the availability of many different classes of molecule that target these pathways specifically, thus allowing the development of rational therapeutic combinations on the basis of mechanism.

An important aspect of B-cell malignancy is that it is possible in most subtypes to obtain adequate numbers of fresh, viable primary malignant cells for *in vitro* genetic, proteomic and functional assessment. However, it is important to take into account the role of the tumour microenvironment. In CLL for example, cells within the proliferation centres within the lymph nodes, bone marrow and spleen have a very different phenotype and drug resistance profile to cells in the peripheral blood[10]. CLL cells within the proliferation centres down-regulate BCL2 and up-regulate other potent anti-apoptotic molecules such as BCL2A1, BCLxL and MCL1, conferring resistance to BCL2-targeted approaches[11-14]. It is therefore necessary in terms of CLL that primary cells are maintained under conditions that mimic those found *in vivo*. However, the optimal culture conditions remain unclear; there are several possible models currently in use, but which (if any) faithfully recapitulate conditions within CLL proliferation centres is not known[15]. Precision medicines that target all anatomical compartments are necessary. An emerging concept in CLL is that several apparently different molecules may result in egress of cells from the lymph nodes into the peripheral blood, where they may become more susceptible to undergo cell death; whether similar events may be seen in other B-cell malignancies is not yet clear. Also, it is also unknown whether there is a distinct stem cell

population for many of the mature B-cell malignancies[16]. There are both genetic and mouse model date to suggest that mature B-cell malignancies may originate in less differentiated haematopoietic stem cell populations[17-19]. Further analysis of mouse models that faithfully recapitulate human B-cell malignancies will be helpful[20].

Nevertheless, most clinical studies proceed without any kind of detailed individual patient assessment. For example, the BTK inhibitor ibrutinib (PCI-32765) has shown significant clinical activity in most cases of CLL, irrespective of signalling from the B-cell receptor (BCR)[21]. If ibrutinib is inhibiting BTK specifically, then it might be anticipated that the drug would only show activity in cases of CLL where BCR signalling remains constitutive. Only about 40% of cases of CLL retain active BCR signalling and yet about 80% of CLL cases show response to ibrutinib, with rapid decreases in lymph node size and concomitant increase in peripheral blood lymphocytes, suggesting that the molecule is interfering with CLL trafficking between lymph node and the periphery. Whether this interesting biological effect is BTK specific or not is not yet known; the kinome targeted by ibrutinib is broad and includes several other kinases with key functions in normal and malignant B-cell physiology. Ibrutinib has been shown to interfere with numerous other signalling pathways (including integrin and chemokine pathways) in CLL, which may explain some of its observed clinical activities[22, 23]. It will be interesting to assess if more specific BTK inhibitors just entering clinical trials will have similar efficacy (Table 1); it is likely that more specific inhibitors may perhaps not induce similar lymphocyte egress from lymph nodes. In comparison, dasatanib, another broad-specificity kinase inhibitor, which also inhibits BTK at nanomolar concentrations, does not show comparable effects in CLL[24]. It is therefore imperative to establish precise modes of action, since only then can rational synergistic combinations be designed.

In contrast, the lack of efficacy of molecules and MAbs targeting the TRAIL pathway in B-cell malignancy, reflects the fact that whilst cell lines may be exquisitely sensitive, all primary B-cell leukaemias and lymphomas are resistant to TRAIL-induced apoptosis, despite cell surface expression of unmutated TRAIL receptors and downstream signalling molecules[25, 26]. Moreover, despite expressing both TRAIL DR4 and DR5 receptors, B-cell malignancies appear only to signal to apoptosis via the DR4 and not the DR5 receptor[27]. The molecular basis of resistance and the differential

signalling via DR4 and DR5 remains unclear. These and many other data highlight the lack of value of preclinical assessment on derived cell lines alone.

There may also be significant problems extrapolating directly data concerning drug sensitivity from one tumour system to another. For example, constitutively activating somatic mutations in the *BRAF* gene have been observed to drive the emergence of a variety of cancers, such as melanoma, thyroid and colorectal cancers[28]. In terms of B-cell malignancies, all cases of hairy cell leukaemia (HCL)[29, 30] and about 3% Myeloma (MM) [31] may also present with *BRAF* mutations. Moreover, the RAF-MEK-ERK pathway may become hyper-activated in other B cell malignancies through BCR engagement[32, 33]. This, together with the fact that solid cancers are normally addicted to the oncogenic activation of this pathway, suggests that BRAF and MEK inhibitors have the potential to provide alternative therapeutic options to B-cell malignancies[34], a suggestion apparently confirmed by an initial clinical report documenting responsiveness to low doses of the BRAF specific inhibitor, vemurafenib in a single patient with HCL[35]. However, such sensitivity to BRAF inhibition in HCL may not be present in all cases. Initial pharmacological studies performed on HCL (before *BRAF* mutations were discovered in this disease) indicated that downstream ERK activation was in fact independent of RAF signalling but sensitive to MEK inhibitors[36]. Confirming these data, preliminary investigations in our laboratory using primary cells from a HCL patient with homozygous $BRAF^{V600E}$ mutation were analysed *in vitro* (Figure 1 A-C). MEK inhibitors (PD325901) affected cell survival, but a BRAF inhibitor (PLX4720) had no major effects. Moreover, neither suppressed ERK phosphorylation. The same inhibitors were able to block ERK phosphorylation in the MM cell line U266, which also harbours *BRAF* mutation. However, neither PD325901 nor PLX4720 affected cell survival in U266, regardless of ERK not being phosphorylated (Figures 1D and 1E). These data show that BRAF inhibitors are not always able to suppress the ERK signalling pathway in blood malignancies, regardless of the presence of the *BRAFV600E* mutation, and that ERK inhibition may affect B-cell survival only in certain conditions. Similarly, in colorectal carcinoma, presence of *BRAF* mutations does not predict sensitivity to vemurafenib, due to the concomitant activation of the EGFR pathway following BRAF inhibition[37]. (In melanoma, the EGFR pathway is not expressed and therefore this mode of bypassing the consequences of BRAF mutation does not exist.) In this instance, concurrent inhibition of both EGFR

and BRAF pathways may be effective. Whether such events occur in HCL is not yet clear but it seems likely that B cell malignancies, despite often having activated RAF-MEK-ERK, may, like solid tumours have other oncogenic pathways that can substitute and confer resistance to BRAF inhibitors. (Data obtained at the Sanger Centre show that *BRAF* mutant cell lines derived from a variety of different primary tumour types show a 10^7 fold difference in sensitivity to BRAF inhibitors: please see for example:

http://www.cancerrxgene.org/translation/Drug/29#scatter_BRAF_29 and

http://www.cancerrxgene.org/translation/Drug/1036#scatter_BRAF_1036 .)

Collectively, these data raise the important point that expression of a specific molecule or of an individual mutation cannot be used to predict response to a functional agent. The overall life-or-death response of a tumour cell population to a given apoptotic stimulus will depend not on one single protein, but rather on the levels of expression (sometimes of different isoforms and post-translationally modified isoforms), possible mutations and altered subcellular localizations of not only the target but many pro- and anti-apoptotic molecules and the integrated response of all their interactions. The degree of "addiction" of malignancies to specific oncogenes will therefore depend on many factors. This is highlighted in the response to various B-cell malignancies *in vitro* to BCL2 inhibition. The BCL2 inhibitor navitoclax/ABT-263 shows considerable *in vitro* activity against all cases of CLL irrespective of *IGHV* gene mutation /FISH abnormalities at nanomolar concentrations [13]. In contrast, MCL and DLBCL samples all expressing BCL2 to comparable levels were much more resistant (Figure 2).

b) Design of rational combinations of precision medicines.

Given that multiple genetic abnormalities are necessary to result in the emergence of the neoplastic phenotype, and given also that cells in different functional or anatomical compartments will have differing phenotypes, and finally given the possible existence of stem cells populations, it is unlikely that a single targeted agent alone will be curative. Despite what appears to be quite remarkable activity of certain new molecules, it is likely that most new agents will be used in combination, due to the rapid development of resistance to single agent therapy.

There are a number of different approaches. As mentioned above, the simplest and quickest approach to getting a product licence is to incorporate the precision medicine with pre-existing chemotherapeutic regimens. The best example in B-cell malignancies has been the incorporation of rituximab into CHOP and FC regimens for DLBCL and CLL respectively. Single agent rituximab has little meaningful clinical activity in either disease. However, its use with chemotherapy has resulted in markedly improved overall survival, especially in DLBCL but also in CLL, and for the most part, with little additional toxicity (reviewed in [38]). The basis for this synergy is not entirely clear but may relate to rituximab-induced down-regulation of key anti-apoptotic regulatory proteins, making cells more sensitive to chemotherapy[39]. In DLBCL, the success of R-CHOP makes introduction of new therapies much more difficult. New, third generation CD20 MAbs with improved immune effector functions such as the type II CD20 MAb obinutuzumab (GA101) are now being compared with rituximab in combinations with CHOP in DLBCL in large, phase III randomized clinical trials[40] (clinical trials ID NCT01287741). However, obinutuzumab, despite its enhanced antibody effector functions, may fail clinical evaluation if the mechanism underlying the observed synergy with chemotherapy is not immune-based. Furthermore, DLBCL expressing low levels of CD20 at the cell surface by flow cytometry (but abundant cytoplasmic CD20 by immunohistochemistry) have been shown to have a poor response to regular rituximab-based chemotherapy and thus a poor prognosis[41]. Newer CD20 MAbs are unlikely to make a significant impact under these conditions. Making viable cell suspensions for flow cytometry from lymph node and other tissue biopsies is usually done only in academic centres, but may nevertheless be a crucial investigation in determining responsiveness to newer agents. This will be of particular importance in therapies that depend on cell signalling rather than simply cell binding for efficacy.

A second approach utilizes “anatomical synergy” using combinations of drugs that work well at different anatomical sites. For example, the CD52 MAb alemtuzumab can effectively deplete CLL cells from blood and bone marrow and induce minimal residual disease (MRD) negative remissions. However, like all other unconjugated MAbs to date, it has been much less effective against “bulky” lymph nodes >5cm in diameter; what limits the efficacy of unconjugated MAbs at such sites is not

clear. Use of high-dose methylprednisolone (HDMP) in combination with alemtuzumab however causes lymphocytes to leave the lymph nodes and enter the peripheral blood where they become more susceptible to MAb[42]. A similar kind of approach may well be possible with ibrutinib. Obinutuzumab, which induces very rapid clearance of the peripheral blood like alemtuzumab but without the loss of residual T-cells, may well be the MAb of choice in this context.

Finally, and perhaps most excitingly, there is the prospect of using combinations of targeted agents in a rational, mechanistic basis. There are few examples of this approach at the current time in B-cell malignancies, although potential synergistic combinations are being defined in solid tumours, as outlined above in malignancies with *BRAF* mutations. A recent example in Burkitt lymphoma, derived from remarkably convergent data from both global high-throughput RNA sequencing and RNA interference screening as well as mouse modelling, has indicated the necessity for constitutive PI3 kinase activation and cyclin D3 activation in most cases[43 44]. The synergy between deregulated MYC, PI3K and cyclin D3 will be targetable therapeutically. Similar synergy between specific BCL6 inhibitors and HDAC and HSP90 inhibitors in DLBCL has also been reported again suggesting a novel therapeutic combination[45]. It is highly likely that other examples will emerge from similar studies in other disease types.

The approach of using relatively non-specific molecules such as HDAC, HSP90 or proteasomal inhibitors for example to sensitise tumour cells to more targeted therapies might find broad therapeutic application. This pharmacological approach is exemplified by the sensitization of B-cell malignancies to TRAIL mediated apoptosis using low doses of HDAC inhibitors. Most primary B-cell malignancies are resistant to TRAIL-mediated apoptosis[25] and much work has been performed in an attempt to overcome the resistance pharmacologically. Various approaches have been used. Nanomolar levels of class 1 type HDACi (much lower than those necessary to induce apoptosis) enhance the recruitment of the adapter protein FADD to the death-initiating signalling complex (DISC), facilitating apoptosis[46]. Comparable sensitization can potentially also be achieved with proteasomal inhibitors[47]. The approach of combining TRAIL agonists and sensitizing small molecules merits further attention and a clinical trial using such combinations has commenced, although in the

context of B-cell lymphoma, a TRAIL-R1 targeted approach would be more effective than one targeting TRAIL-R2 (clinical trial ID NCT00791011 and [48]). Such combinations however are not without considerable potential risks, which may be difficult to predict. For example, concerns about using TRAIL-targeted therapy revolve around possible hepatotoxicity[49]. If concurrent administration of either HDACi or proteasomal inhibitors sensitizes normal as well as malignant cells to TRAIL, then toxicities could well be severe and unacceptable. It is difficult to model this and other combinations adequately in animals; mice for example have only one TRAIL receptor. Studies on isolated human hepatocytes may be of some value, but will not address other systemic toxicities. Similarly, simultaneous inhibition of more than one anti-apoptotic BCL2 family member might have significant systemic toxicities. The lack of suitable animal models and the considerable variability of human tumours, may leave no other option than to proceed cautiously in patients with end-stage disease, for whom no other therapies are available. However, this approach does again mandate exhaustive pre-clinical *in vitro* assessment of individual tumours to ensure that sensitization might be effective in any individual case.

Putting it all together at the academic-pharmaceutical interface.

Progress in our understanding of the molecular causes of many forms of malignancy through the application of whole genome approaches has been remarkable. Some of the precision medicines now available for malignancy are relatively specific and potentially highly effective, but there remains a need to be able to deliver them in a rational and mechanistic fashion. There are now simply too many possible targeted therapies for B-cell malignancies for them all to be assessed empirically. Without careful mechanistic analysis, potentially useful reagents might be discarded and effective molecules used in either a toxic or wasteful manner. Particularly in these financially straightened times, neither outcome is acceptable.

Given that so many molecules fail in development, it is essential that the right patients and the right clinical conditions are used for assessment. Thus far, decisions on the use of targeted therapies in oncology, including decisions on doses and schedules, have been made on pragmatic or financial grounds and not scientific, biological or immunological principles. For example, the doses of

rituximab used in combination with chemotherapy are totally arbitrary. To marry specific tumors with specific combinations of targeted drugs in a mechanistic manner will require more detailed investigation of primary tumours under conditions that mimic those found *in vivo*. The increasing need for functional testing will change not only how primary clinical material is handled at diagnosis, but also perhaps where oncological medicine is practiced. For the leukaemias, this analysis is relatively trivial given the ready availability of tumour cells. For "solid" malignancies however, functional testing will require a major change in how samples are collected surgically and processed. It is unlikely that smaller medical centres will be able to perform the necessary tests. Such assays cannot be performed outside a dedicated, specialised laboratory. How and where such assays should be performed and how quality control of such assays should be assessed remains unclear. The development and assessment will demand much closer integration of clinical and laboratory scientists.

Greater involvement of industry in this process, working closely with academic centres, is also essential in order to move beyond the empirical introduction of precision medicines. For example, at the time of writing (September 2012), most of the agents in Table 1 are being assessed increasingly in combination with regular chemotherapeutic combinations in large Phase 3 clinical trials with little or no assessment of relevant biomarkers. Inevitably, there will be some "winners" and some "losers" in this process. In contrast, as outlined above, we suggest that the process of clinical drug development should be much more biologically and immunologically oriented from the outset, using primary clinical material to define unambiguously mechanisms of action and to define potential synergistic mechanism-based combinations at any early stage of development. Such combinations could be then taken into the clinic in small, "proof-of-principle" studies with detailed mechanistic monitoring. It is, for example, quite conceivable that for some B-cell malignancies, concurrent BCL2, BTK and PI3Kδ inhibition along with obinutuzumab might be most effective and minimally toxic. The rational development of such multi-agent therapy with several different precision medicines (perhaps from different companies) would require much more open relationships between the pharmaceutical industry and academia. Ideally, academia should be involved in the design and assessment of early

phase single agent and combination clinical studies. How such combination therapy with multiple precision medicines might be funded if successful is quite another matter.

We now have some very specific weapons in the fight against cancer and are likely to have many more in the coming years. We must now collectively instigate the means whereby these are delivered in an appropriate fashion, rather than using the “hit-or-miss” strategies of the last century.

References

1. Walker, I. & Newell, H. Do molecularly targeted agents in oncology have reduced attrition rates? *Nat Rev Drug Discov* **8**, 15-6 (2009).
2. Wierda, W.G. et al. Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. *J Clin Oncol* **28**, 1749-55 (2010).
3. Anthony, R.M., Kobayashi, T., Wermeling, F. & Ravetch, J.V. Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature* **475**, 110-3 (2011).
4. Georgakis, G.V. et al. Activity of selective fully human agonistic antibodies to the TRAIL death receptors TRAIL-R1 and TRAIL-R2 in primary and cultured lymphoma cells: induction of apoptosis and enhancement of doxorubicin- and bortezomib-induced cell death. *Br J Haematol* **130**, 501-10 (2005).
5. Dyer, M.J., MacFarlane, M. & Cohen, G.M. Barriers to effective TRAIL-targeted therapy of malignancy. *J Clin Oncol* **25**, 4505-6 (2007).
6. Punt, C.J. & Tol, J. More is less -- combining targeted therapies in metastatic colorectal cancer. *Nat Rev Clin Oncol* **6**, 731-3 (2009).
7. Hambley, T.W. & Hait, W.N. Is anticancer drug development heading in the right direction? *Cancer Res* **69**, 1259-62 (2009).
8. Salmon, S.E. et al. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. *N Engl J Med* **298**, 1321-7 (1978).
9. Alberts, D.S. et al. In-vitro clonogenic assay for predicting response of ovarian cancer to chemotherapy. *Lancet* **2**, 340-2 (1980).
10. Burger, J.A. Nurture versus nature: the microenvironment in chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program* **2011**, 96-103 (2011).
11. Schmid, C. & Isaacson, P.G. Proliferation centres in B-cell malignant lymphoma, lymphocytic (B-CLL): an immunophenotypic study. *Histopathology* **24**, 445-51 (1994).
12. Masir, N. et al. The expression of Bcl-2 by proliferating cells varies in different categories of B-cell lymphoma. *Histopathology* **56**, 617-26 (2010).
13. Vogler, M. et al. Concurrent up-regulation of BCL-XL and BCL2A1 induces approximately 1000-fold resistance to ABT-737 in chronic lymphocytic leukemia. *Blood* **113**, 4403-13 (2009).
14. Willimott, S. & Wagner, S.D. miR-125b and miR-155 contribute to BCL2 repression and proliferation in response to CD40 ligand (CD154) in human leukemic B-cells. *J Biol Chem* **287**, 2608-17 (2012).
15. Hamilton, E. et al. Mimicking the tumour microenvironment: three different co-culture systems induce a similar phenotype but distinct proliferative signals in primary chronic lymphocytic leukaemia cells. *Br J Haematol* (2012).
16. Kelly, P.N., Dakic, A., Adams, J.M., Nutt, S.L. & Strasser, A. Tumor growth need not be driven by rare cancer stem cells. *Science* **317**, 337 (2007).
17. Kikushige, Y. et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. *Cancer Cell* **20**, 246-59 (2011).
18. Vicente-Duenas, C. et al. Expression of MALT1 oncogene in hematopoietic stem/progenitor cells recapitulates the pathogenesis of human lymphoma in mice. *Proc Natl Acad Sci U S A* **109**, 10534-9 (2012).
19. Weigert, O. & Weinstock, D.M. The evolving contribution of hematopoietic progenitor cells to lymphomagenesis. *Blood* (2012).
20. Klein, U. et al. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell* **17**, 28-40 (2010).

21. Advani, R.H.S., J. P.; Smith, S. M.; et al. The Btk Inhibitor PCI-32765 Is Highly Active and Well Tolerated In Patients (Pts) With Relapsed/Refractory B Cell Malignancies: Final Results From A Phase I Study *Annals of Oncology (Suppl.)* **22,** , 135-135 (2011).
22. Herman, S.E. et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* **117**, 6287-96 (2011).
23. de Rooij, M.F. et al. The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia. *Blood* **119**, 2590-4 (2012).
24. Amrein, P.C. et al. Phase II study of dasatinib in relapsed or refractory chronic lymphocytic leukemia. *Clin Cancer Res* **17**, 2977-86 (2011).
25. MacFarlane, M. et al. Mechanisms of resistance to TRAIL-induced apoptosis in primary B cell chronic lymphocytic leukaemia. *Oncogene* **21**, 6809-18 (2002).
26. Rubio-Moscardo, F. et al. Characterization of 8p21.3 chromosomal deletions in B-cell lymphoma: TRAIL-R1 and TRAIL-R2 as candidate dosage-dependent tumor suppressor genes. *Blood* **106**, 3214-22 (2005).
27. Natoni, A. et al. TRAIL signals to apoptosis in chronic lymphocytic leukaemia cells primarily through TRAIL-R1 whereas cross-linked agonistic TRAIL-R2 antibodies facilitate signalling via TRAIL-R2. *Br J Haematol* **139**, 568-77 (2007).
28. Davies, H. et al. Mutations of the BRAF gene in human cancer. *Nature* **417**, 949-54 (2002).
29. Tiacci, E. et al. BRAF mutations in hairy-cell leukemia. *N Engl J Med* **364**, 2305-15 (2011).
30. Xi, L. et al. Both variant and IGHV4-34-expressing hairy cell leukemia lack the BRAF V600E mutation. *Blood* **119**, 3330-2 (2012).
31. Chapman, M.A. et al. Initial genome sequencing and analysis of multiple myeloma. *Nature* **471**, 467-72 (2011).
32. de Gorter, D.J., Vos, J.C., Pals, S.T. & Spaargaren, M. The B cell antigen receptor controls AP-1 and NFAT activity through Ras-mediated activation of Ral. *J Immunol* **178**, 1405-14 (2007).
33. Ramsey, L.B., Vegoe, A.L., Miller, A.T., Cooke, M.P. & Farrar, M.A. Tonic BCR signaling represses receptor editing via Raf- and calcium-dependent signaling pathways. *Immunol Lett* **135**, 74-7 (2011).
34. Packer, L.M. et al. Nilotinib and MEK inhibitors induce synthetic lethality through paradoxical activation of RAF in drug-resistant chronic myeloid leukemia. *Cancer Cell* **20**, 715-27 (2011).
35. Dietrich, S. et al. BRAF inhibition in refractory hairy-cell leukemia. *N Engl J Med* **366**, 2038-40 (2012).
36. Kamiguti, A.S. et al. Regulation of hairy-cell survival through constitutive activation of mitogen-activated protein kinase pathways. *Oncogene* **22**, 2272-84 (2003).
37. Prahallad, A. et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **483**, 100-3 (2012).
38. Dyer, M.J. Safety and efficacy of ofatumumab in patients with fludarabine and alemtuzumab refractory chronic lymphocytic leukaemia. *Therapeutic Advances in Hematology* **3**, 199-207 (2012).
39. Alas, S. & Bonavida, B. Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl-2 and sensitization to cytotoxic drugs. *Cancer Res* **61**, 5137-44 (2001).
40. Mossner, E. et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with

enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood* **115**, 4393-402 (2010).
41. Johnson, N.A. et al. Diffuse large B-cell lymphoma: reduced CD20 expression is associated with an inferior survival. *Blood* **113**, 3773-80 (2009).
42. Pettitt, A.R. et al. Alemtuzumab in combination with methylprednisolone is a highly effective induction regimen for patients with chronic lymphocytic leukemia and deletion of TP53: final results of the national cancer research institute CLL206 trial. *J Clin Oncol* **30**, 1647-55 (2012).
43. Schmitz, R. et al. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* (2012).
44. Sander, S. et al. Synergy between PI3K Signaling and MYC in Burkitt Lymphomagenesis. *Cancer Cell* **22**, 167-79 (2012).
45. Cerchietti, L.C. et al. BCL6 repression of EP300 in human diffuse large B cell lymphoma cells provides a basis for rational combinatorial therapy. *J Clin Invest* (2010).
46. Inoue, S., Harper, N., Walewska, R., Dyer, M.J. & Cohen, G.M. Enhanced Fas-associated death domain recruitment by histone deacetylase inhibitors is critical for the sensitization of chronic lymphocytic leukemia cells to TRAIL-induced apoptosis. *Mol Cancer Ther* **8**, 3088-97 (2009).
47. Liu, F.T. et al. Bortezomib blocks Bax degradation in malignant B cells during treatment with TRAIL. *Blood* **111**, 2797-805 (2008).
48. MacFarlane, M., Kohlhaas, S.L., Sutcliffe, M.J., Dyer, M.J. & Cohen, G.M. TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies. *Cancer Res* **65**, 11265-70 (2005).
49. Volkmann, X. et al. Increased hepatotoxicity of tumor necrosis factor-related apoptosis-inducing ligand in diseased human liver. *Hepatology* **46**, 1498-508 (2007).
50. Harrison, C. Trial watch: BTK inhibitor shows positive results in B cell malignancies. *Nat Rev Drug Discov* **11**, 96 (2012).
51. Woyach, J.A., Johnson, A.J. & Byrd, J.C. The B-cell receptor signaling pathway as a therapeutic target in CLL. *Blood* **120**, 1175-84 (2012).
52. Herman, S.E. & Johnson, A.J. Molecular Pathways: Targeting Phosphoinositide 3-Kinase p110-Delta in Chronic Lymphocytic Leukemia. *Clin Cancer Res* **18**, 4013-8 (2012).
53. Roberts, A.W. et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J Clin Oncol* **30**, 488-96 (2012).
54. Walensky, L.D. From mitochondrial biology to magic bullet: navitoclax disarms BCL-2 in chronic lymphocytic leukemia. *J Clin Oncol* **30**, 554-7 (2012).
55. Davids, M.S. & Letai, A. Targeting the B-cell lymphoma/leukemia 2 family in cancer. *J Clin Oncol* **30**, 3127-35 (2012).
56. Illidge, T.M. Obinutuzumab (GA101)--a different anti-CD20 antibody with great expectations. *Expert Opin Biol Ther* **12**, 543-5 (2012).
57. Salles, G. et al. Phase 1 study results of the type II glycoengineered humanized anti-CD20 monoclonal antibody obinutuzumab (GA101) in B-cell lymphoma patients. *Blood* **119**, 5126-32 (2012).
58. Jak, M. et al. CD40 stimulation sensitizes CLL cells to lysosomal cell death induction by type II anti-CD20 mAb GA101. *Blood* **118**, 5178-88 (2011).
59. Ivanov, A. et al. Monoclonal antibodies directed to CD20 and HLA-DR can elicit homotypic adhesion followed by lysosome-mediated cell death in human lymphoma and leukemia cells. *J Clin Invest* **119**, 2143-59 (2009).

Table legend

This table is by no means an exhaustive list of new precision medicines for B-cell malignancies but serves to illustrate the diversity of some of the newer molecules that have shown considerable efficacy in recent phase I clinical studies.

Figure legends

Figure 1. Effects of BRAF and MEK inhibitors on malignant B-cell survival.

(A) HCL primary cells were treated with 2µM PD325901 (second graph) or 0.6µM PLX4720 (third graph) for 48 hours. Graphs show representative PI staining. Numbers indicate percentage of sub-G1 events (cell death).

(B) Sequence analysis of the BRAF gene in these cells, showing V600E mutation.

(C) Western blot showing inhibition of ERK phosphorylation in the presence of PD325901 but not PLX4720 (non-phosphorylated ERK used as control).

(D) U266 cells were treated with 2µM PD325901 (second graph), 0.6µM PLX4720 (third graph) for 48 hours. Graphs show representative PI stainings. Numbers indicate percentage of SubG1 events (cell death).

(E) Both PD325901 and PLX4720 inhibited ERK phosphorylation, as shown by FACS analysis with a phospho-ERK1/2 specific antibody.

Figure 2. Sensitivity of primary malignant B-cells to the BCL2-inhibitor ABT-737

Malignant B-cells were isolated from patients with Chronic Lymphocytic Leukemia (CLL), Mantle Cell Lymphoma (MCL) and Diffuse Large B-cell Lymphoma (DLBCL). Purified cells were incubated with different concentrations of ABT-737 in RPMI medium supplemented with 10 % FCS for 4 hours. Apoptosis was assessed by exposure of phosphatidylserine, staining with AnnexinV-FITC and propidium iodide and flow cytometry. Each line in the graph represents cells isolated from one individual patient.

Table 1: New precision medicines for B-cell malignancies, September 2012.

Target	Molecule	Company links / preclinical publications	References / Comments
BTK inhibitors	**Ibrutinib** **AVL-292** **ONO 4059**	http://www.pharmacyclics.com/clinical_trial_btk_pcyc_pci32765.html http://www.celgene.com/research/kinase-inhibitors.aspx	Reviewed in [50] and [51]. Single agent ibrutinib has shown considerable activity in CLL with redistribution of CLL cells from lymph nodes and into the peripheral blood. Low toxicity allows prolonged administration. Complete remissions appear to be rare however. From the limited data presented at various meetings it appears that the efficacy of more BTK specific inhibitors may be less than ibrutinib.
PI3KD **inhibitors**	**GS-1101**	http://www.gilead.com/pr_1690087	Reviewed in [52]. Egress of CLL cells from lymph nodes similar to that seen with ibrutinib has been observed with PI3KD inhibitors.
BCL2 inhibitors	**Navitoclax (ABT-263)** **ABT-199 / GDC-0199**	http://www.biooncology.com/pipeline-molecules/bcl-2/index.html	Reviewed in [53], [54] and [55] ABT-199 has higher affinity for BCL2, is more specific for BCL2 than navitoclax, and appears not to induce BCLxL induced thrombocytopenia. In early clinical studies, ABT-199 has been reported to induce tumour lysis in some patients with CLL
CD20	**GA101 /** **Obinutuzumab**	http://www.biooncology.com/pipeline-molecules/ga101/index.html	Reviewed in [56]. See also [57], [58] and [59].
Antibody-drug conjugates	**Various MAbs of different specificity linked to various cytotoxic molecules**	http://www.seagen.com/product_pipeline.php http://www.biooncology.com/research-education/adc/current- research/index.html http://www.pfizer.com/files/news/asco/inotuzumab_fact_sheet.pdf	

Figure 1

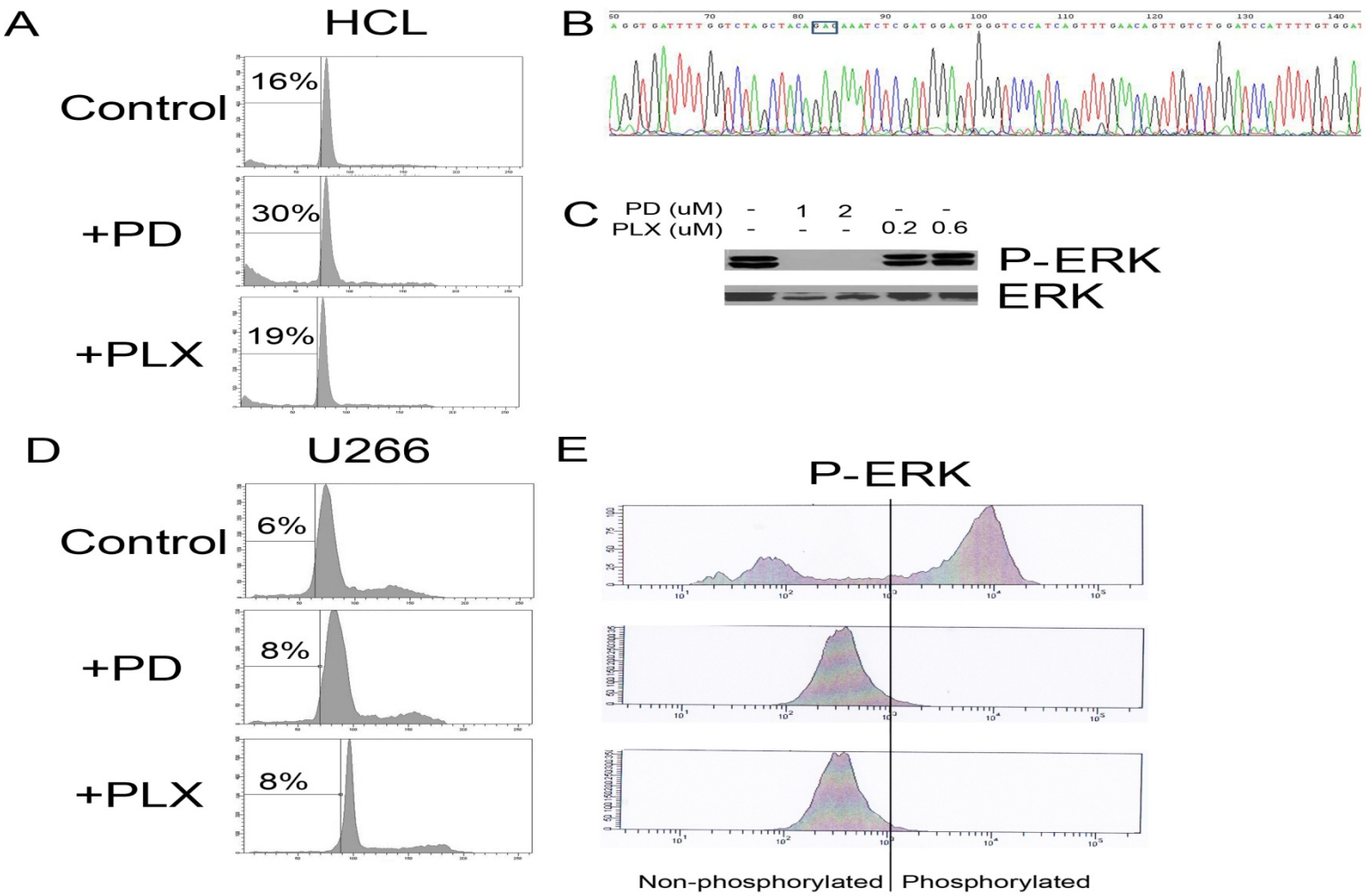
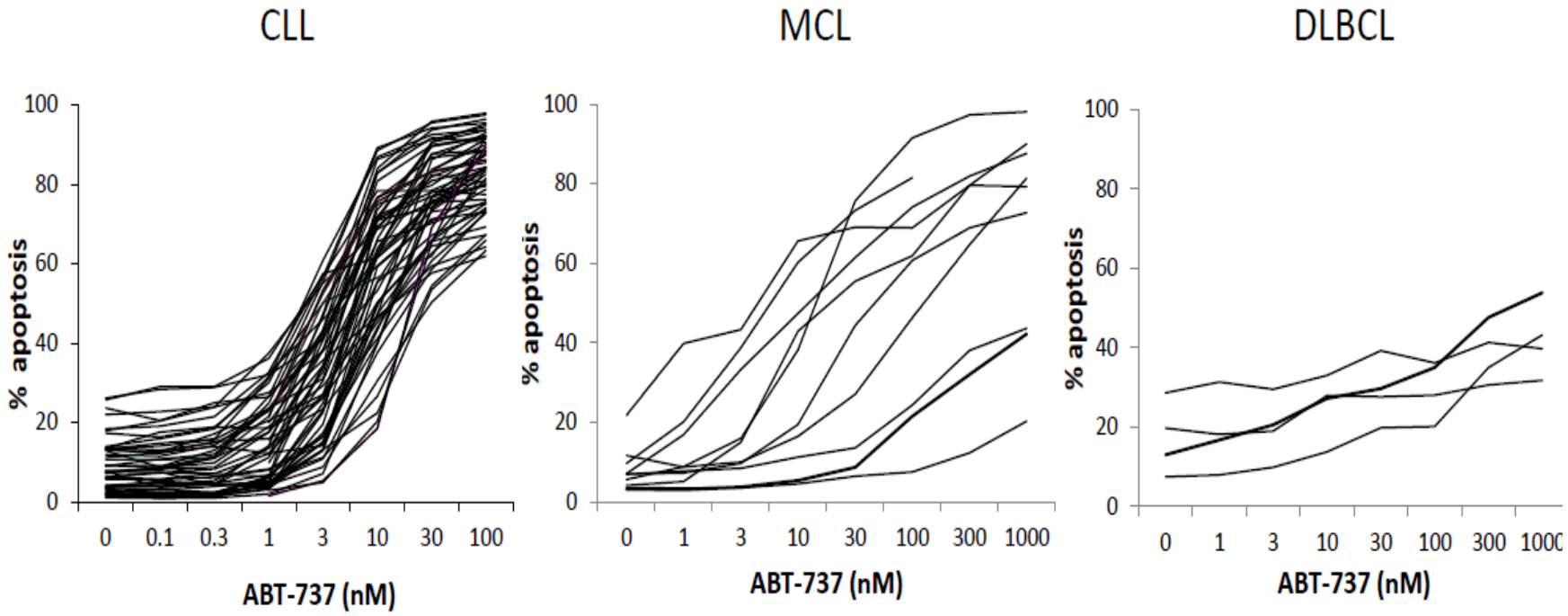

A
HCL
Control
16%
+PD
30%
+PLX
19%
B
C
PD (uM) - 1 2 - -
PLX (uM) - - - 0.2 0.6
P-ERK
ERK
D
U266
Control
6%
+PD
8%
+PLX
8%
E
P-ERK
Non-phosphorylated
Phosphorylated

Figure 2.


CLL
MCL
DLBCL
% apoptosis
100
80
60
40
20
0
0 0.1 0.3 1 3 10 30 100
0 1 3 10 30 100 300 1000
ABT-737 (nM)