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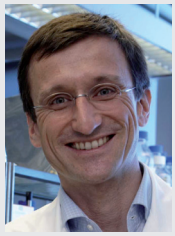

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"…compelling preclinical data support the rationale for the incorporation of chemosensitizing agents in the treatment of refractory acute lymphoblastic leukemia."

The treatment of relapsed childhood acute lymphoblastic leukemia (ALL) remains a major problem in pediatric oncology today. With second-line treatment, approximately a third of the patients can be cured [1–3]. Currently, the best approach to identify patients with high-risk disease is by assessment of the molecular response to chemotherapy [4,5] and to salvage treatment [1]. Intervention with experimental therapy is now possible for patients with persistence of minimal residual disease. Concerted efforts should be fostered to accelerate incorporation of new agents into current treatment regimens.

Improved preclinal modeling of drug-resistant disease

With the explosion of knowledge in cancer biology, and the growing number of promising new agents, major improvements in our capability to triage and optimize candidate approaches must be implemented. This is particularly relevant for orphan diseases in pediatric oncology, where drug development will be immediately jeopardized by competing early-phase clinical trials. Despite the new regulations, incentives for the pharmaceutical industry to develop new agents for pediatric oncology remain limited. Academic programs have been initiated, such as the 'Innovative Therapies for Childhood Cancer' consortium in Europe [6], and the Pediatric Preclinical Testing Program (PPTP) in North America [7], in an attempt to improve prioritization of new agents for clinical evaluation. In collaboration with the ALL Berlin–Frankfurt–Münster (BFM) Study Group, we have decided to build a preclinical triage platform by modeling the patient population that will be eligible for experimental therapeutic intervention in the next generation of ALL treatment protocols, taking advantage of xenotransplantation of primary human ALL cells (ALL primografts) [8–12]. We have now constituted a bank of primografts derived from patients with very high-risk (VHR) ALL (*de novo* resistant), and with heavily pretreated relapsed or refractory ALL. Preliminary results from our validation studies identified the typical genetic lesions in ALL primografts using genome-wide approaches [9], which remain remarkably stable upon serial passage into immunodeficient mice [13]. Therefore, we think that concerted efforts should be undertaken to use this experimental system in order to generate better translational research platforms. Banks of renewable ALL primograft samples can be established to model the clinical setting. By increasing our testing capability on a large number of samples from patients that are in need of experimental therapy, we expect significant advances with preclinical research, which should translate into better strategies for clinical investigation.

New approaches for chemosensitization in ALL

Given the diversity of genetic lesions detected in ALL, it will be crucial to

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identify common denominators for therapeutic targeting. It will also be of advantage to use agents that are likely to be successfully developed for adult indications. New approaches for chemosensitization would meet such requirements. Multiple hallmarks of cancer are linked with mitochondrial dysfunction, such as impaired apoptosis, insensitivity to antigrowth signals, decreased autophagy and metabolic reprogramming [14,15]. Promising approaches are now being developed to target cancer-associated mitochondrial dysfunction and reactivate cell death programs that could have a great impact on the treatment of resistant disease. We, and others, have shown that a blockade of the mitochondrial apoptotic response [8,16] and hyperactivation of AKT and mTOR signaling [17,18] contribute to glucocorticoid resistance in ALL. Mitochondrial apoptosis is controlled by BCL2 family members, whereby the interplay between pro- and anti-apoptotic BCL-2 family proteins can determine the susceptibility of the cells to undergo apoptosis. BH3-only proteins that mainly comprise the critical interaction domain between pro-and anti-apoptotic BCL2 proteins can trigger apoptosis in different ways. New therapeutic agents are being developed that mimic the BH3 domain of BH3-only proteins. We have observed potent antileukemic single-agent activity using the currently best-characterized small-molecule BH3 mimetic, ABT-737 [19], and its orally available derivative, ABT-263 [8,20–22]. There is a rationale for a therapeutic window for ABT-737, because the therapeutic effect appears to be related to a specific type of profile of BCL2 family members at the level of the mitochondria, a feature that may be used for predicting the response to this chemosensitizer [23]. Moreover, the response to ABT-737 was selectively stronger in tumor cells than in cells from normal tissue [24]. *In vitro*, synergy may be difficult to assess for this agent, as it may trigger very effective apoptosis once a threshold concentration is reached. *In vivo*, using xenograft models, ABT-737 potentiated the effect of a three-drug regimen, with dexamethasone, L-asparaginase and vincristine [25], and delayed leukemia progression in combination with L -asparaginase, topotecan, vincristine or etoposide [21]. Provided reliable predictive markers can be established to select patients that will respond to this drug, addition of ABT-263 to a multidrug regimen with a second-line chemotherapy agent should be investigated with priority. Extending the preclinical testing phase of different combinations of drugs with ABT-737 to a panel of primografts that is representative of the patient population with resistant disease will be a prerequisite to identify reliable biomarkers and refine the trial design for this type of chemosensitization therapy.

> "Our preliminary results indicate that obatoclax may provide more effective chemosensitization than mTOR inhibitors."

Increasing evidence suggests that resistance to ABT-737 could be mediated by MCL1, an antiapoptotic BCL2 family member to which ABT-737 can not bind [26,27]. The pan-BCL-2 antagonist, obatoclax, which was shown to disrupt the interaction between MCL-1 and pro-apoptotic Bak [28], induced apoptosis in cocultures of ALL primografts on human mesenchymal stromal cells, with IC_{50} concentrations below 1 µM [8]. We observed that subcytotoxic concentrations (50–150 nM) of obatoclax were sufficient for powerful resensitization of B-cell-precursor (BCP) VHR-ALL cells to daunorubicin, vincristine or cytarabine. Obatoclax, but not ABT-737, also restored the response to dexamethasone completely. Chemosensitization by obatoclax was highly effective *in vivo* [8], and could be confirmed in independent samples from heavily pretreated patients with relapsed and refractory BCP-ALL [Walti R, Bonapace L, Bourquin J-P, Unpublished Data]. Unexpectedly, steroid resensitization did not require mitochondrial apoptosis but, instead, was strictly dependent on rapid induction of autophagy and necroptosis. Necroptosis was recognized as a form of programmed cell death after activation of the death receptor pathway with TNF in the context of defective caspase activity [29,30]. This pathway was proposed to serve as a salvage cell-death mechanism to control lymphoid cell proliferation in response to infectious agents capable of apoptosis inhibition [31]. Necroptosis depends on the function of RIP1 kinase and the deubiquitinase CYLD, known to modulate RIP1 function [29,32], which we confirmed to be critical for the effect of obatoclax. Since this agents acts selectively at subcytotoxic concentrations in resistant ALL cells, it could actually improve the therapeutic window in a situation where alternatives are often to increase treatment intensity.

> "...it is likely that we will achieve biologically active drug levels in pediatric patients at a tolerable dose level."

It will be important to better characterize the mechanisms that prime resistant cells to autophagy-dependent cell death, and to understand how obatoclax can switch between induction of apoptosis and necroptosis. Subcytotoxic concentrations of obatoclax lead to the disruption of an interaction between MCL1 and the autophagy regulator Beclin-1, providing a possible mechanism for autophagy induction. Moreover, combination of obatoclax with dexamethasone resulted in inhibition of mTOR. Interestingly, rapamycin has also been identified as a glucocorticoid sensitizer in ALL [18]. We discovered that steroid sensitization by rapamycin was also mediated in an autophagy-dependent manner [8]. However, the mechanism of action of obatoclax must be different from mTOR inhibitors, because mTOR inhibition was not observed for the combination of obatoclax with nonglucocorticoid chemotherapeutic agents. Our preliminary results indicate that obatoclax may provide more effective chemosensitization than mTOR inhibitors. It will, therefore, be important to compare obatoclax side-by-side with new agents modulating mTOR, including rapalogs [12], BEZ-235 [33] and inhibitors of the mTOR kinase domain [9], on a larger number of resistant ALL cases, which is possible with our xenograft model.

First pediatric trials with obatoclax mesylate

Chemosensitizing agents are now entering clinical evaluation for childhood ALL. The Children's Oncology Group has initiated a Phase I study to establish the safety of one dose of obatoclax in combination with vincristine, doxorubicin and dexrazoxane (clinicaltrial.gov identifier: NCT00933985). In collaboration

with the international BFM Study Group, we have developed a study protocol to evaluate the safety of a 5-day course of dexamethasone, combined with obatoclax infusions every other day. In adults with chronic lymphocytic leukemia, pharmacokinetic studies indicate that at the recommended Phase II dose peak plasma concentrations of obatoclax can be reached, exceeding the range of 100–150 nM, which was optimal for chemosensitization *in vitro* [34]. Thus, it is likely that we will achieve biologically active drug levels in pediatric patients at a tolerable dose level. Dose-limiting toxicity in adults was mostly infusion related and resulted in reversible neurologic symptoms [34–36]. Our secondary objective will be to obtain evidence for biological activity *in vivo* by monitoring treatment response at the single-cell level by flow cytometry, and by detection of characteristic necroptotic cell-death features using electron microscopy. Options for subsequent Phase II development include the incorporation of the combination of obatoclax with dexamethasone as an investigational window for patients in first relapse, and the design of an experimental multidrug regimen for consolidation of patients with insufficient multidrug-resistant response to relapse treatment. Besides obatoclax, other promising strategies will hopefully enter clinical phase testing in pediatrics. ABT-263 is currently evaluated in adults [20]. Based on interesting preclinical data by the PPTP consortium [35], this agent should also be evaluated in refractory childhood ALL. We expect the Phase I study by the Dana Farber Cancer Institute (NCT00874562), which assesses the biological response to rapamycin in combination with steroids in relapsed ALL, to stimulate further clinical evaluation. In conclusion, compelling preclinical data support the rationale for the incorporation of chemosensitizing agents in the treatment of refractory ALL. In addition to the use of monoclonal antibodies as single agents (e.g., blinatumomab, NCT00560794), incorporation of small molecules as chemosensitizers, in our opinion, hold great promise, not only to improve the outcome for relapse and refractory ALL patients, but also with the hope to reduce toxicity of current ALL treatment. Clinical trials with obatoclax, which is particularly interesting owing to its broad profile of chemosensitizing activity, are now being initiated, which will hopefully lead to more effective regimens for the treatment of resistant disease in the not-too-distant future.

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References

1 Bader P, Kreyenberg H, Henze GH *et al.* Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J. Clin. Oncol.* 27(3), 377–384 (2009).

2 Gaynon PS, Angiolillo AL, Carroll WL *et al.* Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983–2002: a Children's Oncology Group Report. *Leukemia* 24(2), 285–297 (2009).

3 Tallen G, Ratei R, Mann G *et al.* Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. *J. Clin. Oncol.* 28(14), 2339–2347 (2010).

4 Conter V, Bartram CR, Valsecchi MG *et al.* Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood* 115(16), 3206–3214 (2010).

5 Schrauder A, Stanulla M, Flohr T *et al.* Prospective evaluation of MRD-kinetics in 274 children with high-risk ALL treated in trial ALL-BFM 2000: insights into development of resistance and impact on further refinement of treatment stratification strategies. *Blood* 585a (2007).

6 Zwaan CM, Kearns P, Caron H *et al.* The role of the 'innovative therapies for children with cancer' (ITCC) European consortium. *Cancer Treat. Rev.* 36(4), 328–334 (2010).

7 Houghton PJ, Morton CL, Tucker C *et al.* The pediatric preclinical testing program: description of models and early testing results. *Pediatric Blood Cancer* 49(7), 928–940 (2007).

8 Bonapace L, Bornhauser BC, Schmitz M *et al.* Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. *J. Clin. Invest.* 120(4), 1310–1323 (2010).

9 Kharas MG, Janes MR, Scarfone VM *et al.* Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL+ leukemia cells. *J. Clin. Invest.* 118(9), 3038–3050 (2008).

10 le Viseur C, Hotfilder M, Bomken S *et al.* In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. *Cancer Cell* 14(1), 47–58 (2008).

11 Liem NL, Papa RA, Milross CG *et al.* Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. *Blood* 103(10), 3905–3914 (2004).

12 Teachey DT, Obzut DA, Cooperman J *et al.* The mTOR inhibitor CCI-779 induces apoptosis and inhibits growth in preclinical models of primary adult human ALL. *Blood* 107(3), 1149–1155 (2006).

13 Schmitz M, Mirkowska P, Breithaupt P *et al.* Leukemia-initiating cells are frequent in very high risk childhood acute lymphoblastic leukemia and give rise to relatively stable phenotypes in immunodeficient mice. *Blood* 114(22), 86a (2009).

14 Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. *Nat. Rev. Drug Discov.* 51(5), 476–489 (2010).

15 Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 13(6), 472–482 (2008).

16 Bachmann PS, Gorman R, Mackenzie KL, Lutze-Mann L, Lock RB. Dexamethasone resistance in B-cell precursor childhood acute lymphoblastic leukemia occurs downstream of ligand-induced nuclear translocation of the glucocorticoid receptor. *Blood* 105(6), 2519–2526 (2005).

17 Bornhauser BC, Bonapace L, Lindholm D *et al*. Low-dose arsenic trioxide sensitizes glucocorticoid-resistant acute lymphoblastic leukemia cells to dexamethasone via an Akt-dependent pathway. *Blood* 110(6), 2084–2091 (2007).

18 Wei G, Twomey D, Lamb J *et al*. Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. *Cancer Cell* 10(4), 331–342 (2006).

19 Oltersdorf T, Elmore SW, Shoemaker AR *et al*. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 435(7042), 677–681 (2007).

20 Tse C, Shoemaker AR, Adickes J *et al*. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* 68(9), 3421–3428 (2008).

21 High LM, Szymanska B, Wilczynska-Kalak U *et al*. The Bcl-2 homology domain 3 mimetic ABT-737 targets the apoptotic machinery in acute lymphoblastic leukemia resulting in synergistic *in vitro* and *in vivo* interactions with established drugs. *Mol. Pharmacol.* 77(3), 483–494 (2010).

22 Lock R, Carol H, Houghton PJ *et al*. Initial testing (stage 1) of the BH3 mimetic ABT-263 by the pediatric preclinical testing program. *Pediatric Blood Cancer* 50(6), 1181–1189 (2008).

23 Del Gaizo Moore V, Schlis KD, Sallan SE, Armstrong SA, Letai A. BCL-2 dependence and ABT-737 sensitivity in acute lymphoblastic leukemia. *Blood* 111(4), 2300–2309 (2008).

24 Buron N, Porceddu M, Brabant M *et al*. Use of human cancer cell lines mitochondria to explore the mechanisms of BH3 peptides and ABT-737-induced mitochondrial membrane permeabilization. *PloS One* 5(3), e9924 (2010).

25 Kang MH, Kang YH, Szymanska B *et al*. Activity of vincristine, L-ASP, and dexamethasone against acute lymphoblastic leukemia is enhanced by the BH3-mimetic ABT-737 *in vitro* and *in vivo*. *Blood* 110(6), 2057–2066 (2007).

26 Konopleva M, Contractor R, Tsao T *et al*. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell* 10(5), 375–388 (2006).

27 Yecies D, Carlson NE, Deng J, Letai A. Acquired resistance to ABT-737 in lymphoma cells that up-regulate MCL-1 and BFL-1. *Blood* 115(16), 3304–3313 (2010).

28 Nguyen M, Marcellus RC, Roulston A *et al*. Small molecule obatoclax (GX15–070) antagonizes MCL-1 and overcomes MCL-1-mediated resistance to apoptosis. *Proc. Natl Acad. Sci. USA* 104(49), 19512–19517 (2007).

29 Degterev A, Hitomi J, Germscheid M *et al*. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nat. Chem. Biol*. 4(5), 313–321 (2008).

30 Jaattela M, Tschopp J. Caspase-independent cell death in T lymphocytes. *Nat. Immunol.* 4(5), 416–423 (2003).

31 Bell BD, Leverrier S, Weist BM *et al*. FADD and caspase-8 control the outcome of autophagic signaling in proliferating T cells. *Proc. Natl Acad. Sci. USA* 105(43), 16677–16682 (2008).

32 Hitomi J, Christofferson DE, Ng A *et al*. Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* 135(7), 1311–1323 (2008).

33 Serra V, Markman B, Scaltriti M *et al*. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res*. 68(19), 8022–8030 (2008).

34 O'Brien SM, Claxton DF, Crump M *et al*. Phase I study of obatoclax mesylate (GX15–070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia. *Blood* 113(2), 299–305 (2008).

35 Paik PK, Rudin CM, Brown A *et al*. A Phase I study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in solid tumor malignancies. *Cancer Chemother. Pharmacol*. DOI: 10.1007/s00280-010-1265-5 (2010) (Epub ahead of print).

36 Schimmer AD. Apoptosis in leukemia: from molecular pathways to targeted therapies. *Best Pract. Res. Clin. Haematol*. 221(1), 5–11 (2009).