Editorial

The MCL-1 inhibitor S63845: an exciting new addition to the armoury of anti-cancer agents

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Defects in apoptotic machinery have long been recognised as both a significant contributor to cancer development, and as an important mechanism by which tumour cells develop chemotherapeutic resistance. The resistance of multiple malignancies to apoptosis has been attributed to increases in a number of pro-survival BCL-2 family members (e.g., BCL-2, BCL-XL, MCL-1, BCL-W, BFL-1 and BCL-B), which prevent BAX/BAK-mediated mitochondrial outer membrane permeabilisation. Inhibitors targeting these BCL-2 family members have garnered significant interest with the most promising lead being the BH3 mimetic venetoclax (also known as ABT-199, and marketed as Venclexta™ and Venclyxto™), a selective inhibitor of the BCL-2 protein recently approved for 17p deletion chronic lymphocytic leukemia (CLL). In a phase I trial in relapsed or refractory CLL, venetoclax induced a 79% response rate (1) which has subsequently prompted further trials in other haematological malignancies. Despite this success in CLL, venetoclax used as a monotherapy in other haematological malignancies have shown poor response rates (2), mainly due to the reliance of other BCL-2 family members such as MCL-1 for cell survival in these cancers. Indeed, studies using genetic knockout models and RNA interference have demonstrated MCL-1 to be crucial for disease development and progression in acute myeloid leukaemia (AML) (3), MYC-driven lymphomas (4), and multiple myeloma (5), and a mechanism of venetoclax resistance in these cancers (6). Indirect approaches to target MCL-1 through transcriptional repression (7,8) or post-translational degradation (9) have recently been developed. However, direct targeting strategies with obatoclax, an inhibitor of MCL-1 and also BCL-2 and BCL-XL, induced neuronal toxicity (10,11). More recently, a reported MCL-1-selective inhibitor termed A-1210477 (12) displayed *in vitro* activity against multiple myeloma cells (13); however, these anti-cancer effects appear likely to result from combination of both targeting MCL-1 and off-target effects (14).

Recent work published by Kotschy *et al.* in *Nature*, characterised a new, selective MCL-1 inhibitor, S63845 (15). In a manner similar to other BCL-2 family inhibitors, such as venetoclax, S63845 binds to the BH3-binding groove of MCL-1, and thus acts as a 'BH3 mimetic' to block the pro-survival activity of MCL-1 through blocking its interactions with BAX and BAK. S63845 binds to MCL-1 with high affinity (Kd of 0.19nM) with no discernible binding to the other BCL-2 members, BCL-2 or BCL-XL. Indeed, compared to the previously published MCL-1 inhibitor, A-1210477 (12), S63845 has an approximately 20-fold higher affinity for human MCL-1. This, combined with the binding of A-1210477 to serum proteins reducing its bioavailability, an issue not apparent with S63845, translated into S63845 having a 1,000-fold increase in potency in killing multiple myeloma cells compared to A-1210477. Like A-1014077, S63845 displayed selectivity in killing cancer cells that rely on MCL-1 for survival, such as H929 multiple myeloma cells, but not other cancer cells that rely on BCL-2 or BCL-XL for survival, confirming its on-target

effects.

S63845 induced death of cancer cell lines with known reliance on MCL-1, displaying classical hallmarks of apoptosis that were dependent on caspases and BAX/BAK-mediated mitochondrial outer membrane permeabilisation. Chemotherapeutics can induce BAX/BAK-mediated cell death by increasing levels of BH3-only proteins, Noxa and Bim which inhibit BCL-2 family members such as MCL-1, thus allowing BAX/BAK to permeabilise the mitochondrial membrane to facilitate apoptosis (16). MCL-1 inhibitors such as S63845 and A-1210477 not only perform the roles of Noxa and Bim in binding and inhibiting MCL-1, but also free these BH3-only proteins to inhibit other BCL-2 family members to potently induce apoptosis. The on-targets effects of S63845 were confirmed by genetic deletion of MCL-1 in cell lines and S63845 sensitivity correlated with the impact of the MCL-1 specific BIM2A peptide. Screening of S63845 across multiple cell lines from several malignancies confirmed MCL-1-dependent multiple myeloma, lymphoma and AML cell lines are highly sensitive to S63845 (with IC50 <1 μM). Conversely, resistant cell lines (with IC50 >1 μM) revealed an inverse correlation between S63845 sensitivity and BCL-XL expression levels, in agreeance with previous reports, and potentially providing a biomarker of patient response to S63845 (17). Notably, pre-clinical studies with venetoclax revealed a similar trend with high levels of either MCL-1 or BCL-XL sufficient to elicit venetoclax resistance implicating redundancy between the three proteins and suggesting targeting of multiple BCL-2 proteins may be the best strategy (18).

Despite S63845 being ineffective as a monotherapy in numerous solid tumours, Kotschy *et al.* performed combinational studies with numerous targeted therapies including HER2 inhibitors, EGFR inhibitors, MEK inhibitors and RAF inhibitors and found that in all cases these kinase inhibitors enhanced S63845 efficacy (15). This is consistent with the previously described effects of these kinase inhibitors in sensitizing tumour cells to other BH3-mimetic drugs targeting BCL-2 and/or BCL-XL (16). Indeed, numerous previous studies have demonstrated that at clinically achievable doses, these kinase inhibitors, and many other anti-cancer agents, do not kill cells directly, but cause cell stress that triggers apoptosis. This therapy-induced apoptosis often occurs via activation of BH3-only proteins like BIM, explaining why BH3 mimetics such as S63845 can effectively enhance this process (16). Correlations between S63845 efficacy and BCL-XL expression levels were also observed in a number of solid tumour cell lines supporting its applicability as a marker of S63845 efficacy.

Excitingly, when Kotschy *et al.* tested S63845 against Eμ-Myc lymphoma mouse models they observed potent anti-cancer activity with 70% of mice effectively cured of disease. Similarly, S63845 reduced tumour volume of both multiple myeloma and AML cell line xenografts grown in the flanks of mice. With the reported role of MCL-1 in normal homeostasis, in particular haematopoiesis, and genetic studies suggesting MCL-1 is essential for the survival of many cell types (19-21) it is surprising that at doses capable of tumour eradication there was no observable toxicity on healthy tissues (19-21). Indeed, the temporal dampening of MCL-1 using S63845 may circumvent the pathologies associated with complete knockout of gene function. Alternatively, MCL-1 may have BH3 binding groove-independent functions which are only uncovered by genetic studies. Irrespective of this, while S63845 is well tolerated in mice, it must be noted that this agent has a 6 fold higher affinity for human MCL-1 over mouse MCL-1, and so its potential progression to clinical trials in humans will need to be approached with caution. Indeed, while the studies of Kotschy *et al.* suggests a suitable therapeutic window exists for the application of S63845 in the treatment of a number of different malignancies, this is gauged via the use of human cancer cells in mice. Thus, the differential affinity of S63845 for human and mouse MCL-1 would suggest this therapeutic window in patients is likely to be substantially smaller than the current studies would suggest.

Collectively, Kotschy *et al.* provides strong evidence for the on-target activity of the MCL-1 inhibitor S63845, as well its potential use across a number of haematological malignancies including myeloma, AML and lymphoma. Whilst the authors showed S63845 exhibits *in vivo* anti-tumour activity, it would be interesting to observe whether a similar response occurs using more physiologically relevant orthotopic models of cancer such as syngeneic models of AML including those driven by AML1-ETO9a and MLL-AF9 and most importantly primary patient derived xenografts (PDXs) in immunocompromised mice. Clonal heterogeneity observed in primary patient samples is far more diverse with up to ten distinct sub-clones reported in some cases compared with the few observed in cell lines utilised in this study (22). One might expect the vast diversity of the clonal population to exhibit a heterogeneous response in the context of MCL-1 inhibition and potentially enrich for a resistant clone. The authors

in this study utilised AML patient samples to test whether MCL-1 inhibition could recapitulate the nanomolar potency observed in AML cell lines *in vitro*. Of the number of samples tested, a select few patient samples treated with S63845, displayed a potential therapeutic window when compared with healthy $CD34^+$ donor cells. Future studies should extend these studies and analyse the effect of S63845 of primary patient cells co-cultured on autologous stromal layers. Some patient cells resistant to S63845 were sensitive to venetoclax highlighting the need for assays such as BCL-2 protein profiling, which has provided an accurate *in vitro* guide for patient response in Phase II trials assessing venetoclax monotherapy in relapsed/refractory AML (2).

More importantly, the use of primary patient samples is essential to ascertain the impact of MCL-1 inhibition on the cancer stem cell (CSC) population. As a pool of disease maintenance and drug resistance, targeting CSCs is thought to be crucial for achieving deep and sustained molecular remissions. Pre-clinical studies have suggested leukemic stem cells to be reliant on BCL-2 for survival (23,24). Thus it would be interesting to know whether S63845 can effectively target the CSC population *in vitro* and *in vivo*.

In conclusion, Kotschy *et al.* have developed a potent BH3 mimetic highly selective for MCL-1. Initial *in vitro* and *in vivo* validation studies have shown efficacy in targeting cancer cell lines dependent on MCL-1 as a monotherapy and in combination with additional targeted therapies solid tumour cell lines. This data reaffirms the importance of MCL-1 as a therapeutic target across a number of malignancies and the use of assays such as BCL-2 protein profiling will allow patient stratification to select for patients that are likely to respond best to S63845 treatment (low BCL-XL, high MCL-1). We look forward to the results of pharmacokinetic/pharmacodynamic studies that will hopefully facilitate the progression of S63845 into clinical trials for the treatment of MCL-1-dependent cancers. Successful outcomes in this work may add an important agent to the armoury to treat these cancers which currently have a poor prognosis.

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Footnote

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