Pro-survival Bcl-2 Proteins are Modifiers of MYC-VX-680 Synthetic Lethality

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Targeting the MYC Oncogene

The transcription factor encoded by the myelocytomatosis oncogene (MYC) is deregulated by distinct means in different human cancers. Aberrations include chromosomal translocation, amplification, mutation, enhancer activation and post-translational mechanisms that lead to MYC protein accumulation [1]. It is perhaps the most commonly deregulated oncoprotein and is linked to most of the hallmarks of cancer [2]. Yet, despite decades of research, targeting MYC's transforming function is still somewhat enigmatic. Without a kinase domain, it has proven difficult to target with small molecule inhibitors and since MYC is an intracellular protein, it is currently inaccessible to large molecules such as antibodies. A promising alternative approach, that has yet to be fully realized clinically, is to target MYC overexpressing cells with compounds that are synthetic lethal to MYC. Cancers that may benefit from successful synthetic lethal targeting of MYC include various leukemias and lymphomas where MYC deregulation plays a significant role in malignancy [3-5].

MYC Synthetic Lethality with AURKB Inhibition

Synthetic lethal interactions arise when specific oncogenic events impart overdependence on the integrity of specific cellular pathways and functions. Interference with these dependencies can result in dramatic anti-tumor effects. Examples of synthetic lethal interactions with MYC include cell death induced by activation of the TNF receptor superfamily member DR5 (encoded by TNFRSF10B) [6], dependence on the non-essential amino acid glutamine [7], pharmacological inhibition of CDK1 [8], PIM1 [9] or CHK1/2 kinases [10,11] and genetic depletion of AMPK-related kinase 5 [12] or the core spliceosome component, BUD31 [13]. However, the most well studied synthetic lethality with MYC may be the enhanced sensitivity of MYC overexpressing cells to Aurora kinase B (AURKB) inhibition [14-16]. AURKB, along with the proteins INCENP, Borealin and Survivin, form the chromosomal passenger protein complex (CPPC). The kinase activity of AURKB, the catalytic subunit of the complex, is spatially and temporally regulated during mitosis through association with the other components and this is guided by the combined activities of multiple other kinases [17]. A functioning CPPC is required for key events in mitosis, including safeguarding proper chromosome-microtubule attachment, spindle assembly checkpoint activation and cytokinesis. Accordingly, transient inhibition of AURKB can induce both mitotic arrest with apoptosis in MYC expressing cells alongside the induction of polyploidy from failed cytokinesis in cells that escape the earlier fate [14].

Recently, MYC has been linked to enhanced expression of genes impacting centrosome function and chromosomal instability (CIN) arising from errors in chromosome segregation [18]. Key proteins enabling the synthetic lethality with MYC and AURKB inhibition may be encoded by a subset of these centrosomal genes or even the cumulative effect of upregulating many genes that alter centrosome function, as MYC appears to do. Furthermore, the CPPC targets AURKB activity to the inner centromere where it plays a key role in regulating spindle microtubule attachment and the integrity of the spindle assembly checkpoint (SAC) [17]. Since, knockdown with inhibitory RNAs of any of AURKB, INCENP or Survivin, all components of the CPPC, produces a synthetic lethal
effect with MYC [14], one might speculate that the over-
dependence of MYC expressing cells on robust CPPC
function relates to proper kinetochore integrity and
spindle assembly in conjunction with the centrosome,
which would be enabled by a functional SAC.

However, events arising post chromosome segregation
may also play a role in the synthetic lethality of MYC
overexpressing tumors to AURKB inhibition. The in
vivo treatment of genetically engineered murine models
(GEMMs) of both T and B cell lymphoma with the pan-
AURK inhibitor, VX-680, leads not just to apoptosis,
but also the emergence of polyploidy and induction of
autophagy [14]. So, it would seem that in vivo, some VX-
680 treated cells do succeed in completing anaphase,
but fail to complete cytokinesis, therefore becoming
multinucleate. If and how long these cells can persist in
vivo, their contribution to tumor evolution and selective
means for targeting their removal are all active areas of
investigation.

Isogenic Cell Lines for the Study of MYC-
VX-680 Synthetic Lethality

Human retinal pigment epithelial (RPE) cell lines
have proven a valuable tool for studying synthetic lethal
interactions with MYC [8,14,18]. RPE cells immortalized
by the ectopic expression of hTERT are relatively
refractory to aurora kinase inhibition. However, adding
ectopic expression of MYC (RPE-MYC cells) confers robust
sensitivity to these cells. After just 3 days of a transient VX-
680 treatment, RPE-MYC cells are induced to undergo two
distinct types of cell death (Figure 1A) [14]. The first to arise
occurs in approximately 30-40% of cells and is an apoptotic
cell death that follows arrest of cells in pro-metaphase. The
BH3-only protein, BCL2L11 (BIM) contributes to events
that lead eventually to the cleavage of effector caspases and
PARP. However, not all RPE-MYC cells succumb to early
apoptosis. Some cells complete mitosis but fail to complete
cytokinesis. Polyploidy results and these multinucleate
cells come to dominate the culture dish for a short period

Figure 1: Synthetic lethal cell death with MYC and AURKB inhibition. (A) Retinal pigment epithelial
(RPE) cells are refractory to the pan-AURK inhibitor, VX-680, unless they are engineered to overexpress the MYC
oncogene. MYC expressing cells undergo two kinds of cell death in response to VX-680. First, a subset of cells
arrest at the SAC. These cells often have multiple centrosomes that have seeded spindle assembly. Apoptotic death
ensues. Other cells complete anaphase but fail to complete cytokinesis. These cells develop into multinucleate cells
that eventually succumb to autophagy-associated cell death. (B) Hypothetical model for how the ability to bypass
autophagy-associated death may contribute to tumor relapse. Even if AURKB inhibition causes in vivo apoptosis and
lethal autophagy, rare cells that have acquired the ability to bypass autophagy and recommence reductive division
may go on to reseed tumor formation. Whether such a mechanism contributes to recurrence in AURKB inhibitor-
treated cancer is currently speculative.
before they too succumb to cell death. This cell death is not apoptosis, however. The wave of cell death of polyploid cells is associated with a robust induction of autophagy. The autophagy-associated cell death is close to absolute in polyploid RPE-MYC cells and it is this high sensitivity that lends this cell system to the search for modifying events. Escape and the recommencement of reproductive mitosis occurs in approximately 10 in one million RPE-MYC cells [19]. The escape appears to occur through commencement of multipolar cell divisions that reduce the number of nuclei, as the emergent RPE-MYC colonies contain diploid cells with a cell size and nuclear morphology resembling naïve parental cells prior to treatment with VX-680. It is unknown if these cells have acquired further genomic or epigenomic changes and escaping cells are not resistant to re-challenge with VX-680, so the emergence of post-treatment RPE-MYC colonies is not associated with loss of drug response.

A Survey for Modifiers of MYC-VX-680 Synthetic Lethality

Various human cancer-derived cell lines also respond to transient VX-680 treatment with the same two phases of cell death, early apoptosis and autophagy-associated death of polyploid cells [14]. However, there is a differential ability to resume cell proliferation in different human cancer cells, not the near absolute loss of this ability as in RPE-MYC cells. One hypothesis as to why this could be the case is that additional oncogenic events that occur alongside MYC deregulation confer some degree of resistance. This has, until now, not been directly tested. Given the important role that polyploid cells have been ascribed in cancer evolution [20], this is an important line of investigation not only with respect to AURK inhibitor treatment, but for many other therapeutic approaches as well where polyploid cells are induced.

In order to investigate if the oncogenes and tumor suppressor genes that are often altered alongside MYC in cancer can influence the VX-680 phenotype, we constructed a series of isogenic RPE-MYC cell lines. Cells were engineered to overexpress oncogenes and dominant negative forms of tumor suppressors alongside MYC [19]. Then, a transient VX-680 treatment assay was carried out to measure the effects of these manipulations on MYC-VX-680 synthetic lethality. One strength of this approach was that it is an assay of both forms of cell death in response to AURK inhibition. Apoptosis was assayed after 3 days of VX-680 treatment and polyploid cell survival was assayed 3 days after this.

Pro-survival Bcl-2-family Members Prolong Polyploid Cell Survival

Several unexpected findings arose from the study of MYC-VX-680 synthetic lethality in the isogenic cell series. The tumor suppressor p53 is mutated in many cancers where MYC is overexpressed and MYC-induced apoptosis can be both dependent and independent of p53 activation, depending on context [21]. However, previous work has linked p53 function to Aurora kinases [22,23], so one might expect that a dominant negative version of p53 might disrupt the apoptotic response to VX-680 in RPE-MYC cells. This did not happen as the mitotic arrest and apoptosis of diploid RPE-MYC cells was not significantly altered with expression of a dominant negative form of p53, even though this dominant negative p53 did interrupt the apoptotic response to doxorubicin. In RPE-MYC cells, the wave of apoptotic cell death contributing to MYC-VX-680 synthetic lethality does not appear to be p53-dependent.

The mitochondrial apoptotic cascade ultimately leads to the activation of the pro-apoptotic Bcl-2 family proteins Bax, Bak or Bok, mitochondrial outer membrane permeabilization, cytochrome C release and the activation of effector caspases [24]. Another finding of interest revealed by the isogenic cell series was the surprising degree to which VX-680-induced apoptosis in MYC expressing cells was refractory to manipulations that have been found to inhibit MYC-induced apoptosis in other systems, especially the overexpression of pro-survival Bcl-2 family proteins. Overexpression of either Bcl-2 or Bcl-xL was not able to alter the percentage of cells that succumbed to apoptosis after 3 days of VX-680 treatment [19]. One can conclude from this that the cascade of events that leads to apoptotic cell death in VX-680 treated RPE-MYC cells is distinct from apoptosis mediated by MYC in other contexts. For example, MYC overexpressing cells will undergo apoptosis upon loss of growth factor stimulation by serum withdrawal, and this can be blocked by Bcl-2 overexpression [25,26]. In addition, MYC-CDK1 synthetic lethal apoptosis can be blocked by Bcl-2 co-expression in Rat1a cells [8]. Therefore, the MYC-VX-680 synthetic lethal apoptosis appears to be distinct from MYC-mediated apoptosis in other contexts.

Perhaps most intriguing was that even though the pro-apoptotic Bcl-2 family proteins were not able to alter the course of the apoptotic cell death, both Bcl-2 and Bcl-xL were able to prolong the survival of polyploid cells following VX-680 treatment [19]. Polyploid RPE-MYC, engineered to express either of the two pro-survival Bcl-2 proteins, even came to form a monolayer that covered the culture dish following treatment. Eventually, these cells did succumb to autophagy-associated cell death, despite substantial delay of this eventuality. In addition, the emergence of colonies in long-term culture was enabled by Bcl-2 approximately 3-fold. This suggests that inhibition of the autophagy-associated death of polyploid cells may also enable these cells to eventually undergo reductive divisions and resume proliferation, a mechanism that may impact cancer recurrence following treatment (Figure 1B).
Although the mechanism by which Bcl-2 and Bcl-xL enable survival of polyploid RPE-MYC cells following VX-680 treatment does not involve the inhibition of mitochondrial apoptosis, it was found that interaction with a BH3-only protein localized to the endoplasmic reticulum mediates this ability. Beclin 1 (BECN1, ATG6), is a well-described binding partner for Bcl-2 family pro-survival proteins in this organelle [27-29]. The Bcl-2*G145A mutant, which lacks the ability to bind Beclin 1, was not able to repress autophagy. Either was a Bcl-2 fusion protein targeted solely to mitochondria. Apparently, the Bel-2 / Beclin 1 interaction at the endoplasmic reticulum is the required activity.

**Implications for AURKB-inhibitory Therapy**

The combination of AURK inhibitor plus a BH3 mimetic drug is one that holds promise. In breast cancer cell lines, VX-680 and the BH3 mimetic ABT-737 combine to produce an anticancer effect [30]. Similarly, AURKB inhibition and blockade of Bcl-xL function by the BH3 mimetic, ABT-263, produces a robust anti-cancer effect in cell lines from distinct cancers [31]. The effectiveness of combined therapy, however, has been previously attributed solely to enhanced apoptosis. Our findings suggest that the blockade of pro-survival Bcl-2 members from engaging Beclin 1 is an additional mechanism by which combination treatment could work. Combined therapy could overcome the intrinsic resistance conferred by Bcl-2 to autophagy-associated death in certain malignancies to enhance the potency of AURKB inhibition.

Although the BH3-mimetic, Venetoclax (Venclexta®, ABT-199), is used clinically for the treatment of certain hematopoietic cancers, a clinically approved inhibitor of AURKB is not yet available. Toxicities and the lack of adequate efficacy have limited the use of AURKB inhibitors. However, development of rationale combination therapies and predictive biomarkers would certainly allow drug development efforts targeting AURKB to bear fruit. One malignancy where a combined therapy approach might prove successful is diffuse large B-cell lymphoma (DLBCL) where both MYC and Bcl-2 are commonly activated and aneuploid/polyploid cells are suggested to play a role in relapse [32].

We have tested Bcl-2 and Bcl-xL in our assays [19], but Mc1 and Bfl-1/A1 also interact directly with Beclin 1 [27,33]. Since similar inhibitory actions toward Beclin 1-induced autophagy might be mediated by Mc1 and Bfl-1/A1, the target specificity of BH3 memetics used in combination therapy will have to be considered. Tissue and cell-type specific interactions with Beclin 1 may differ.

**Future Directions**

By no means was the isogenic cell screen for modifiers discussed here a comprehensive screen. We tested a limited number of “best-estimate” cancer genes for their ability to modify MYC-VX-680 synthetic lethality. Hundreds of proteins both prepare the cell for and participate in mitosis. Additional proteins that can act as therapeutic targets to promote or enhance synthetic lethality with MYC may be discovered through RNAi or CRISPR/Cas9-based screens. The RPE-MYC screening system, with its combined robust apoptotic response and the induction of polyploidy upon AURKB inhibition, may be ideal for screening new targets and compounds. What may ultimately prove the ideal target for MYC synthetic lethality could be a specific regulator of AURKB solely during early mitosis or alternatively, only during cytokinesis. Although this has not yet been achieved, such specificity might be accomplished by targeting upstream regulators of the CPPC.

**References**


