In our recent manuscript we demonstrated that apilimod, a drug originally brought into the clinic for inflammatory diseases, has highly potent activity against all subtypes of B-cell non-Hodgkin lymphoma in vitro. We also observed antitumor activity in in vivo models of B-NHL as a single agent and in combination with the targeted therapies rituximab and anti-CD274/PD-L1 at drug concentrations achievable in humans. As a result, we recently initiated a multicenter trial (NCT02594384) to test apilimod’s efficacy in B-NHL patients.

The sensitivity of B-NHL cells to apilimod raises important questions about underlying mechanisms. Apilimod was previously shown to inhibit PIKFYVE (phosphoinositide kinase, FYVE-type zinc finger containing). We found that the activity of apilimod toward PIKFYVE is exquisitely selective, as no off-target activity was detected across a panel of 456 normal and disease-related protein and lipid kinases. We furthermore isolated an apilimod-resistant mutant of PIKFYVE that renders cells insensitive to this drug.

PIKFYVE is an endosomal lipid kinase that is targeted to endosomes via interactions between its FYVE domain and phosphatidylinositol-3-phosphate (PtdIns3P). At endosomes, PIKFYVE phosphorylates PtdIns3P to generate PtdIns(3,5)P2. PtdIns(3,5)P2 is a defining component of late endosomes and lysosomes and regulates multiple aspects of their function such that PtdIns(3,5)P2 depletion following PIKFYVE inhibition produces a striking swelling of endolysosomal organelles and induces dephosphorylation and translocation of TFEB, the master regulator of lysosomal biogenesis, into the nucleus. These changes coincide with an increase in LysoTracker staining, and an increase in lysosomal gene expression. Whereas these observations suggest that apilimod-treated cells trigger a homeostatic response that is meant to restore endolysosomal function, we also observed an accumulation of unprocessed cathepsins, and increases in SQSTM1/p62 and LC3-II. The build up of immature cathepsins suggests that this homeostatic response is not successful. Indeed, elevated levels of SQSTM1/p62 and LC3-II indicates a deficit in the ability of lysosomes from apilimod-treated cells to clear autophagic cargoes.

Interestingly, the disruption of lysosome function appears to drive a noncanonical form of cell death. Whereas apilimod-treated B-NHL cells exhibit upregulation of the apoptotic marker ANXA5/annexin V, the death induced by apilimod is not blocked by inhibitors of caspases, cathepsins or necroptosis. This suggests that apilimod is not inducing lysosomal permeabilization in these cells, despite the formation of swollen endolysosomal compartments.

What drives the exquisite sensitivity of B-NHL versus healthy cells to apilom? Our experiments revealed that TFEB expression levels are a major determinant of such sensitivity. B-NHL cells express high levels of TFEB. The cause of high TFEB expression in B-NHL is unclear, although it is noteworthy that TFEB was originally discovered from a B-cell derived cDNA library and may have an unexplored role in normal B-cell physiology. Our experiments in the TFEB-null and apilom-resistant Burkitt lymphoma cell line CA46 further robustly established a role for TFEB expression in determining drug sensitivity, as re-expression of TFEB in these cells drives a dramatic increase in apilom-induced vacuolization and cell death.
To further explore the genetic factors underlying sensitivity of B-NHL cells to apilimod, we performed a genome-wide loss-of-function CRISPR screen to identify genes whose loss confers drug resistance to an otherwise sensitive B-NHL cell line. Remarkably, we identified the lysosome-related genes CLCN7, OSTM1, SNX10 and TFEB as the top factors inducing resistance. CLCN7-OSTM1 form a chloride transporter required for proper lysosomal acidification, and CLCN7 is a TFEB target gene. SNX10 encodes an endosomal sorting nexin of largely unknown function, but the overexpression of SNX10 induces the formation of giant vacuoles. Finally, the discovery that loss of TFEB confers resistance in the screen underscores the importance of this lysosomal master regulator in determining apilimod sensitivity.

Interestingly, loss of either chloride channel component, CLCN7 or OSTM1, is sufficient to confer loss both of vacuolization and increased LysoTracker staining in response to apilimod treatment. This effect is accompanied by near-total resistance to the drug. We therefore hypothesize that apilimod-induced vacuolization may be driven by osmotic deregulation arising from chloride channel overactivity, and that B-NHL cell death lies downstream of this effect. Nuclear translocation of TFEB and transcription of TFEB-target genes may further stress cells with drug-impaired endo/lysosomal function, representing a combined effect that is the driving force behind B-NHL cell death (Fig. 1). How inhibition of PIKfyve activates the CLCN7-OSTM1 transporter and regulates TFEB remains to be determined, but these downstream events appear to be independent. We observed that TFEB still becomes dephosphorylated in cells that have deletions in CLCN7 or OSTM1 (unpublished results) and that vacuole formation and expansion of the acidified compartment still occur in the absence of TFEB. Elucidating the underlying mechanisms will both further delineate the PIKfyve functions and potentially reveal novel mechanisms of lysosomal homeostatic regulation.

In conclusion, we have elucidated disruption of lysosomal homeostasis as a novel anticancer mechanism for the killing of B-NHL tumor cells. Targeting PIKfyve represents a novel therapeutic strategy to treat B-NHL, and apilimod represents a first-in-class kinase inhibitor approach to treating this disease. The drug has been extensively profiled in previous clinical trials and is well tolerated. In our ongoing clinical trial, we are formally defining a maximal tolerated dose for apilimod in B-NHL patients and monitoring safety, pharmacokinetics/pharmacodynamics, and preliminary efficacy.

Disclosure of potential conflicts of interest
S.G., N.B., S.L., C.C., P.B., M.H., and H.L. are employees at LAM Therapeutics. T.X. is on the LAM Therapeutics advisory board. S.M.F. is a consultant to LAM Therapeutics. J.R. is a Director of LAM Therapeutics. LAM Therapeutics is the owner of apilimod patents.

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