Cancers develop as a result of cancerous proteins being activated or tumor suppressor proteins being inactivated. The transcription factor c-Myc is an example of a cancerous protein: this transcription factor is overexpressed in a majority of human cancers, so targeting it has long been viewed as a potential approach to cancer therapy.

c-Myc usually works in partnership with another transcription factor, called MAX, and a variety of cofactors. c-Myc and MAX form a dimer that binds to DNA and then recruits and interacts with one or more cofactors to promote the transcription of certain target genes (Lin et al., 2012; Thomas et al., 2015). However, c-Myc is also involved in many cellular processes in healthy cells, and this has complicated efforts to target it for therapeutic applications.

There are at least three reasons why it is difficult to target c-Myc and/or its cofactors (Fletcher and Prochownik, 2015). First, many cancers are caused by specific mutations, and it is possible to design small molecules that target specific mutation sites. However, in the case of c-Myc, cancer is caused by overexpression, which makes targeting more difficult. Second, we could target some of the cofactors, but we do not know how or where they bind to c-Myc. Third, like c-Myc itself, some of these cofactors also have essential roles in normal cells. However, we can exploit the fact that c-Myc binds to DNA in regions of high acetylation.

In 2011 researchers at the Dana-Farber Cancer Institute, Harvard Medical School and other institutes in the US decided to explore if it was possible to target c-Myc by inhibiting a protein called BRD4 (Delmore et al., 2011). This protein belongs to the BET (short for bromodomain and extra-terminal) subfamily of human bromodomain proteins. Delmore et al. focused on BRD4 for two reasons: it is known to bind to DNA in regions of high acetylation; and it is known to regulate gene transcription by recruiting a protein called P-TEFb, which is one of the cofactors involved in the gene transcription network regulated by c-Myc (Figure 1). They found that JQ1, a small molecule that inhibits BRD4, can inhibit the progression of certain kinds of blood cancer both in vitro and in vivo (Delmore et al., 2011).

In 2015, as part of the Reproducibility Project: Cancer Biology, Kandela et al. published a Registered Report (Kandela et al., 2015) which
explained in detail how they would seek to replicate selected experiments from Delmore et al. The results of these experiments have now been published as a Replication Study (Aird et al., 2017). The Replication Study broadly confirms that JQ1 can repress the progression of certain blood cancers by targeting cofactors of c-Myc.

In the original work, Delmore et al. measured gene expression and cell proliferation in vitro (in blood cancer cell lines) before and after treatment with JQ1, and observed that treatment with JQ1 resulted in significant changes in the transcription of many (but not all) of the genes in the c-Myc-related gene transcription network: 88 genes were down-regulated by a factor of two or more, and 25 were up-regulated by a similar factor. In particular, the transcription of c-Myc was down-regulated. They also evaluated the effect of JQ1 in vivo (in a mouse model of blood cancer) and showed that treatment with JQ1 decreased the amount of c-Myc protein in the cells, reduced the proliferation of cancer cells and increased survival.

In the replication study, Aird et al. observed that JQ1 treatment down-regulated the transcription of c-Myc in vitro and increased the overall survival of the mouse model in vivo, which is consistent with the original paper. However, the decrease of tumor burden (as measured by bioluminescence) in vivo was not statistically significant, possibly because many of the control mice had to be euthanized before the pre-specified endpoint because of disease progression and high tumor burden.

Aird et al. also used an enantiomer of JQ1 that cannot inhibit BET proteins as a control. Although this enantiomer does not affect the transcription of c-Myc, it does somewhat surprisingly display pharmacological activity in mouse
models (possibly caused by the enantiomer unexpectedly interacting with or metabolizing various molecules in vivo).

Other research groups have found that the inhibition of BET bromodomain proteins has suppressed the expression of c-Myc in a range of cell lines and in mouse models for other blood cancers (Dawson et al., 2011; Dawson and Kouzarides, 2012). A number of molecules that inhibit BET bromodomain proteins are also undergoing clinical trials (Berthon et al., 2016). However, treatment with JQ1 did not affect the transcription of c-Myc in other cell lines, including embryonic stem cells and certain cells in lung cancers, so certain cancers will be resistant to JQ1 treatment (Horne et al., 2015; Lockwood et al., 2012; Shimamura et al., 2013).

It is not understood why JQ1 affects some but not all of the genes in the c-Myc-related gene transcription network. Are unknown cofactors involved in the transcription, or are variations in the acetylation state important? Whatever the answer, it may be possible to combine BET bromodomain inhibitors with other small molecules for cancer therapy.

Moreover, in addition to acetylation, other forms of epigenetic modification (such as DNA methylation and nucleosome remodeling) might be important in the c-Myc-related gene transcription network. Theoretically, any of the molecules or processes in the network could be targeted to curb the oncogenic effects of c-Myc, just as BRD4 can be targeted. And since the targeting of metabolic enzymes has proved effective in mouse tumor models, it might be possible to develop new therapies based on the fact that c-Myc has a role in controlling cellular metabolism (Figure 1; Bonnet et al., 2007; Le et al., 2010; Xiang et al., 2015).

The sheer complexity of the c-Myc-related gene transcription network poses many challenges for researchers, but it also offers many opportunities for the development of more effective and specific therapies for many cancers, including human cancers that are resistant to JQ1.

Note
Ping Gao was one of the reviewers for the Registered Report (Kandela et al., 2015) and the Replication Study (Aird et al., 2017).

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Competing interests: The authors declare that no competing interests exist.

Published 19 January 2017

References
Aird F, Kandela I, Mantis C, Reproducibility Project: Cancer Biology. 2017. Replication Study: BET bromodomain inhibition as a therapeutic strategy to target c-Myc. eLife 6:e21253. doi: 10.7554/eLife.21253
Berthon C, Raffoux E, Thomas X, Vey N, Gomez-Roca C, Yee K, Taussig DC, Rezai K, Roumier C, Herait P, Kahatt C, Quesnel B, Michallet M, Recher C, Lokiec F, Preudhomme C, Dombret H. 2016. Bromodomain inhibitor OTX015 in patients with acute leukemias: a dose-escalation, phase 1 study. The Lancet Haematology 3:e186–e195. doi: 10.1016/S2352-3026(15)00247-1, PMID: 27063977
Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED. 2007. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11:37–51. doi: 10.1016/j.ccr.2006.10.020, PMID: 17222789
Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirgouto E, Doeheker N, et al. 2011. Inhibition of BET recruitment to chromatin as an effective treatment for ALL-fusion leukaemia. Nature 478:529–533. doi: 10.1038/nature10509, PMID: 21964340
Dawson MA, Kouzarides T. 2012. Cancer epigenetics: from mechanism to therapy. Cell 150:12–27. doi: 10.1016/j.cell.2012.06.013, PMID: 22770212
Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilapatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Hefferman TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, et al. 2011. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 146:904–917. doi: 10.1016/j.cell.2011.08.017, PMID: 21889194
Fletcher S, Prochownik EV. 2015. Small-molecule inhibitors of the Myc oncoprotein. Biochimica Et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 1849:525–543. doi: 10.1016/j.bbagrm.2014.03.005, PMID: 24657798
Horne GA, Stewart HJ, Dickson J, Knapp S, Ramsahoye B, Chevassut T. 2015. Nanog requires BRD4 to maintain murine embryonic stem cell pluripotency and is suppressed by bromodomain inhibitor JQ1 together with Lefty1. Stem Cells and Development 24:879–891. doi: 10.1089/scd.2014.0302, PMID: 25393219
Kandela I, Jin HY, Owen K, Reproducibility Project: Cancer Biology. 2015. Registered Report: BET
bromodomain inhibition as a therapeutic strategy to target c-Myc. eLife 4:e07072. doi: 10.7554/eLife.07072, PMID: 26111384

Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, Royer RE, Vander Jagt DL, Semenza GL, Dang CV. 2010. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. PNAS 107:2037–2042. doi: 10.1073/pnas.0914433107, PMID: 2013848

Lin CY, Lovén J, Rahl PB, Paranal RM, Burge CB, Bradner JE, Lee TI, Young RA. 2012. Transcriptional amplification in tumor cells with elevated c-Myc. Cell 151:56–67. doi: 10.1016/j.cell.2012.08.026, PMID: 23021215

Lockwood WW, Zejnullahu K, Bradner JE, Varmus H. 2012. Sensitivity of human lung adenocarcinoma cell lines to targeted inhibition of BET epigenetic signaling proteins. PNAS 109:19408–19413. doi: 10.1073/pnas.1216363109, PMID: 23129625

Shimamura T, Chen Z, Soucheray M, Carretero J, Kikuchi E, Tchaicha JH, Gao Y, Cheng KA, Cohoon TJ, Qi J, Akbay E, Kimmelmann AC, Kung AL, Bradner JE, Wong KK. 2013. Efficacy of BET bromodomain inhibition in Kras-mutant non-small cell lung cancer. Clinical Cancer Research 19:6183–6192. doi: 10.1158/1078-0432.CCR-12-3904, PMID: 24045185

Thomas LP, Wang Q, Grieb BC, Phan J, Foshage AM, Sun Q, Olejniczak ET, Clark T, Dey S, Lorey S, Alicie B, Howard GC, Cawthon B, Ess KC, Eischen CM, Zhao Z, Fesik SW, Tansey WP. 2015. Interaction with WDR5 promotes target gene recognition and tumorigenesis by MYC. Molecular Cell 58:440–452. doi: 10.1016/j.molcel.2015.02.028, PMID: 25818646

Xiang Y, Stine ZE, Xia J, Lu Y, O’Connor RS, Altman BJ, Hsieh AL, Gouw AM, Thomas AG, Gao P, Sun L, Song L, Yan B, Slusher BS, Zhuo J, Ooi LL, Lee CG, Mancuso A, McCaillon AS, Le A, et al. 2015. Targeted inhibition of tumor-specific glutaminase diminishes cell-autonomous tumorigenesis. Journal of Clinical Investigation 125:2293–2306. doi: 10.1172/JCI75836, PMID: 25915584