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EDITORIAL

Some leopards can change their spots: potential repositioning of stem cell reprogramming compounds as anti-cancer agents

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Abbreviations

AML	Acute myeloid leukemia
EMT	Endothelial-mesenchymal transition
GSK-3β	Glycogen synthase kinase-3β
iPSCs	Induced pluripotent stem cells
JAK/STAT3	Janus activated kinase/signal transducer and activator of transcription 3
MAPK	Mitogen activated protein kinase
MEF	Mouse embryonic fibroblast
MEK1	Mitogen-activated protein kinase kinase
TRAIL	Tumor necrosis factor (TNF) α-related apoptosis inducing ligand

Introduction

In the context of drug discovery and development, repositioning is defined as the application of previously characterized compounds in new disease scenarios (Langedijk et al. 2015; Tobinick 2009). Repositioning has also been termed repurposing, re-profiling, re-tasking, or therapeutic switching (Langedijk et al. 2015). This approach has significant advantages compared to traditional drug discovery approaches, because the repositioned compound will already have been characterized in other disease context(s). Repositioned compounds can function in the new disease context via a known target or a novel target mechanism (Fig. 1).

Small molecule compounds have been used to facilitate the generation of induced stem cells (Jung et al. 2014c). These cells are created using techniques that artificially modulate the epigenetic status of target cells (Krause et al. 2015). In addition to small molecule-based approaches, other established methods for producing induced stem cells include nuclear transfer into enucleated oocytes (Gurdon and Wilmut 2011), cell fusion with pluripotent/totipotent stem cells (Do et al. 2007), or the addition of exogenous agents, such as vectors encoding reprogramming transcription factors (Takahashi and Yamanaka 2006), microRNAs (Anokye-Danso et al. 2011), and recombinant proteins (Zhou et al. 2009). Of these approaches, small molecule-based methodologies have received significant attention

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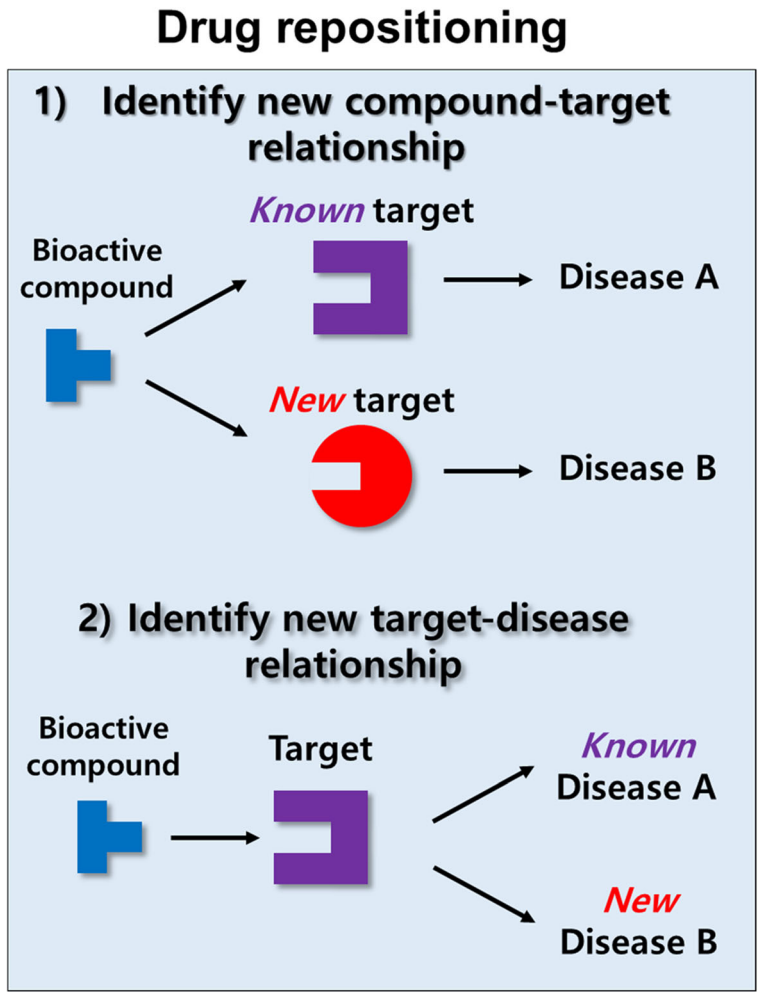


Fig. 1 Schematic illustrating how the repositioning of known bioactive compounds for novel disease applications can provide novel drug targets or reposition the known target in a new disease context

from the research community (reviewed in (Yu et al. 2014)). These methodologies aimed to replace one or more of the classical "Yamanaka" reprogramming transcription factors (Oct-3/4, Sox-2, c-Myc, and Klf4 (Takahashi and Yamanaka 2006)) with small molecules. This approach has value because small molecules possess a number of advantages as tools to induce stem cell phenotypes (reviewed in (Zhang et al. 2012)). In brief, small molecules (classified as less than 800 (Dougherty et al. 2012) or 500 Da (Lipinski 2003)) are relatively cheap to produce and require relatively simple storage and quality control requirements, compared to other reagents such as recombinant proteins or synthetic RNAs. Their molecular weight limit allows oral bioavailability, which is advantageous for subsequent drug development. Moreover, an individual small molecule has the potential to produce numerous effects in the target cell, via binding to multiple protein targets (for example, retinoic acid, which targets different nuclear receptors). Prominent examples of small molecules that are employed in the production of induced stem cells include RepSox, an inhibitor of transforming growth factor-β, which can substitute for Sox-2 in mouse embryonic fibroblast (MEF) reprogramming to induced pluripotent stem cells (iPSCs), and kenpaullone, which can replace Klf4 in MEF reprogramming (Lyssiotis et al. 2011; Ichida et al. 2009). Currently, there are numerous small molecules that can facilitate the iPSC reprogramming process or completely substitute for the Yamanaka transcription factors (reviewed in (Jung et al. 2014c; Yu et al. 2014; Lin and Wu 2015)).

In the case of small molecule-based methodologies for stem cell induction, an ultimate aim is to provide a source of precursor cells that can be used to treat

degenerative diseases, such as Alzheimer's disease or heart failure. Ideally, these small molecules could even be administered as drugs that directly induce tissue repair in vivo (discussed in (Langle et al. 2014)). However, recently, it has become apparent that some of these reprogramming compounds have the potential to be repositioned for pre-clinical development as anti-cancer agents. This may appear counterintuitive, because cancer progression also involves the acquirement of stem cell characteristics (Hernandez-Vargas et al. 2009). However, the target mechanisms of certain cell reprogramming molecules are also relevant for carcinogenesis. Numerous examples have been reported, such as the histone deacetylase inhibitor, valproic acid and the glycogen synthase kinase-3β (GSK-3β) inhibitor, SB-216763 (De Souza and Chatterji 2015; Mazor et al. 2004). A discussion of all of these molecules would be beyond the scope of this commentary. Herein, we focus on two interesting examples: 6-bromoindirubin-3′-oxime (BIO) and 2-(4-morpholinoanilino)-6-cyclohexylaminopurine (reversine).

Reversine: synthetic purine that targets cell division for epigenetic reprogramming and anti-cancer activity

The compound, reversine (Fig. 2), was discovered using combinatorial chemistry and high-throughput screening for small molecule modulators of somatic cell reprogramming (in this case, the conversion of muscle cells into bone-lineage cells, via detection of the osteogenic marker, alkaline phosphatase) (Chen et al. 2004). This synthetic compound is based on the purine chemical motif, which possesses multiple biological activities. For example, triazolopyrimidines (8-azapurines) have applications in cancer and viral chemotherapy (Parker et al. 2004). Reversine treatment induced

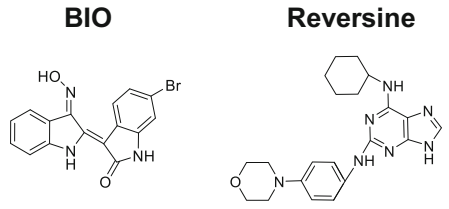


Fig. 2 Chemical structures of 6-bromoindirubin-3′-oxime (BIO) and 2-(4-morpholinoanilino)-6-cyclohexylaminopurine (reversine)

muscle cells to behave as multipotent stem cells, which was validated by the additional finding that reversine treatment also induced muscle cell conversion into fat-lineage cells after culture in adipogenic culture media. The cell reprogramming effect of reversine was confirmed in human cells (Chen et al. 2007). The biological targets of reversine were initially characterized as non-muscle myosin II heavy chain and mitogen-activated protein kinase kinase (MEK1), which were both required for the reprogramming effect. It was also noted that reversine blocked cell cycle progression in treated cells. Subsequently, numerous studies have illustrated that the ability of reversine to induce multipotency in a wide variety of cell types (e.g., macrophages into mesenchymal stem-like cells (Qu and Von Schroeder 2012), muscle stem cells into female germ-like cells (Lv et al. 2012), and preadipocytes into osteogenic cells (Park et al. 2014)). This effect has recently been linked to reversine-mediated activation of Oct4 expression, which is one of the Yamanaka iPSC reprogramming factors (Li et al. 2016).

Further analysis of the biological mechanism of reversine revealed that the active target may not be non-muscle myosin II heavy chain and MEK1, but rather inhibition of aurora kinases A and B, which localize to the centrosome during mitosis and carry out pivotal functions during cell division (Amabile et al. 2009). Inhibition of aurora kinases results in abnormal formation of the mitotic spindle, improper alignment of segregating chromosomes, and reduced phosphorylation of the histone H3 target (D'Alise et al. 2008; Santaguida et al. 2010). These effects also suggest a potential mechanism for reversine-induced stem cell reprogramming: the epigenetic remodeling of chromatin structure.

Aurora kinase is also an anti-cancer target (D'Assoro et al. 2015). Previously characterized aurora kinase inhibitors, such as VX-680, have been used in clinical trials for cancer treatment and shown treatment efficiency (Cheung et al. 2014). The anti-cancer activity of reversine was first demonstrated in a panel of human cancer cell types, such as HeLa cervical carcinoma, PC-3 bladder adenocarcinoma, and primary acute myeloid leukemia (D'Alise et al. 2008; Hsieh et al. 2007). Moreover, reversine was shown to inhibit signaling mediated by focal adhesion kinase (Bijian et al. 2013), which is a key regulator of cancer cell invasion and migration (Avallone et al. 2015). It has been demonstrated that the reversine target, aurora kinase, also

regulates focal adhesion kinase activity (Romain et al. 2014).

Cancer progression is not only dependent upon the cancer cells themselves but also results from a complex communication network involving cancer cells and non-cancer cells, such as stromal fibroblasts and tumor-associated macrophages, which is termed the tumor microenvironment (reviewed in (Junttila and de Sauvage 2013)). This microenvironment can also modulate resistance to chemotherapy (Grigorieva et al. 1998). Consequently, a high-throughput bioluminescence-based screening system was established to identify compounds that can target myeloma cancer cells co-cultured with stromal cells, which models the tumor microenvironment (McMillin et al. 2010). Over 3000 compounds were screened, and interestingly, reversine was one of the best performing compounds and more effective against tumor cells in the presence of stromal cells compared to tumor cell cultures alone. Dramatically, this selectivity was confirmed in vivo. Reversine treatment reduced tumor burden in an infused myeloma mouse model, in which tumor cells interact with bone marrow stromal cells. In contrast, tumor growth from myeloma cells that were transplanted subcutaneously and do not interact with stromal cells was unaffected by reversine treatment. These results validated reversine as an anti-cancer drug that can target tumor microenvironment interactions in vivo to overcome stromal cell effects on cancer cell chemoresistance. This notable anti-cancer feature of reversine was reiterated in a subsequent study. Laser capture microdissection technology was used to generate micro-patterned co-cultures of tumor and stromal cells (Shen et al. 2014). It was observed that the interface between cancer and stromal cells produced marked gene expression and multiple signaling pathway changes in cancer cells, compared to cancer cells that are not in contact with stromal cells. For example, *MMP14*, a metalloproteinase involved in cancer cell invasion, and *TWIST14*, a marker of endothelial-mesenchymal transition (EMT) that also indicates migratory capability, showed increased cancer cell invasion at the stromal interface. Significantly, reversine treatment targeted these cancer cells to decrease expression of metastasis-related genes and was the most effective drug in a panel of known stromal-targeting drugs, such as bortezomib (the first therapeutic proteasome inhibitor; approved for treating multiple myeloma) and resveratrol (a natural product stilbenoid). The effect on stromal interaction was confirmed in a human breast cancer xenograft model, in which reversine treatment reduced tumor size. Histological examination of tumors revealed reduced stromalization in the reversine-treated mice, with reduced collagen staining and less numbers of cells expressing the stromal fibroblast marker, α-smooth muscle actin. Thus, reversine possesses an interesting bioactivity as an anti-cancer drug, because it targets the cellular interactions in the tumor microenvironment in vivo to inhibit tumorigenesis. Overall, the current state of knowledge concerning studies of reversine in cancer is summarized in Box 1. The anti-cancer application of reversine and its analogs has been patented by researchers at Dana-Farber Cancer Institute (US Patent no. 8,466,147, filed 13 June 2013). Next in this commentary, we describe the repositioning of the marine natural product derivative and stem cell modulator, BIO, as an anti-cancer agent.

Box 1: Summary of current knowledge about the use of reversine in cancer research

(1) Induction of autophagy in human follicular thyroid cancer cells via inhibition of Akt/mTOR/p70S6 K-related pathways (Lu et al.)
(2) Preferentially cytotoxic for p53-deficient cancer cells (Jemaa et al.)
(3) Suppresses breast cancer tumor growth and metastasis in vivo by reducing tumor stromalization (collagen deposition, recruit activated stromal cells) (Shen et al.)
(4) Blocking human breast cancer cell proliferation via cell cycle arrest, induction of polyploidy, and apoptosis (Kuo et al.)
(5) Suppression of oral squamous cell proliferation by cell cycle arrest and induction of autophagy via inhibition of Akt/mTORC1 (Lee et al.)
(6) Inhibition of differentiated and undifferentiated thyroid cancer cell proliferation via cell cycle arrest or apoptosis (Hua et al.)
(7) Synergy with aspirin for growth inhibition and apoptosis in human cervical cancers cells (Qin et al.)
(8) Inhibition of focal adhesion disassembly and turnover to reduce breast cancer cell migration via focal adhesion kinase inhibition (Bijian et al.)
(9) Specific anti-cancer effect in various cancer cell lines via cell cycle arrest and induction of apoptosis; not observed in normal fibroblasts (Piccoli et al.)
(10) Inhibition of protein kinase monopolar spindle 1 (MPS1) to preferentially kill tetraploid tumor cells (Jemaa et al.)
(11) Induction of growth arrest and polyploidy in human cancer cell lines via increased expression of p21 (WAF1)/down-regulation of cyclin B and CDK1 (Hsieh et al. 2007)
(12) Inhibition of colony formation in human acute myeloid leukemia (D'Alise et al. 2008)
(13) Modulation of tumor cell-stroma interactions to reduce the development of chemoresistance (McMillin et al. 2010)

BIO: a mollusk-derived compound for stem cell renewal, cardiogenesis, and inhibiting tumorigenesis

The small molecule, BIO ((2′Z,3′E)-6-bromoindirubin-3′-oxime; Fig. 2), was first characterized in 2003 (Meijer et al. 2003). It is a cell permeable derivative of the natural product, 6-bromoindirubin that is produced by predatory rock snails, such as *Hexaplex trunculus*, which was used in ancient times to produce the highly prized "Tyrian purple" dye. BIO was shown to selectively inhibit the multifunctional enzyme, GSK-3β, leading to activation of the Wnt signaling pathway. This pathway maintains the undifferentiated state of stem cells (Willert et al. 2003), and it was shown that BIO treatment produced developmental defects in zebrafish embryos (Meijer et al. 2003). Subsequently, it was demonstrated that BIO treatment could maintain the pluripotency of stem cell cultures and prevent spontaneous cell differentiation (Sato and Brivanlou 2006; Nagai et al. 2014; Holmes et al. 2008). Notably, it was shown that periodic activation of Wnt signaling by BIO treatment enhanced somatic cell reprogramming to pluripotent stem cells using cell fusion methodology (Lluis et al. 2008). GSK-3β inhibition by BIO is also an important component of small molecule-based approaches to derive functional cardiomyocytes from embryonic stem cells and iPSCs, in which it is employed at an early stage to derive mesodermal lineage cells (Naito et al. 2006; Jung et al. 2014c). Interestingly, BIO treatment could also induce proliferation in post-mitotic adult cardiomyocytes, which involved Wnt signaling activation and down-regulation of the cell cycle inhibitor, p27 (cyclin-dependent kinase inhibitor 1B/ Kip1) (Tseng et al. 2006).

Perturbation of Wnt signaling is commonly encountered in cancer cells and is a feature of carcinogenesis (reviewed in (Polakis 2012)). Glycogen synthase kinase-3β is also an anti-cancer target (Li et al. 2015). Thus, although BIO was initially utilized in stem cell biology, the known effect of this compound on Wnt signaling lead to investigations concerning its potential anti-cancer activity. Initial analysis focused on the effects on osteolytic bone lesions in an in vitro model of multiple myeloma cells interacting with bone marrow cells (Gunn et al. 2006). Myeloma cells secrete the Wnt pathway inhibitor, Dickkopf-1, which prevents osteogenesis and induces proliferation in bone marrow mesenchymal stem cells. Thus, treatment with BIO disrupted this pathogenic cycle, resulting in reduced proliferation and osteogenic differentiation in mesenchymal cells. This result indicated that BIO could be developed as a drug to treat osteolytic disease in multiple myeloma. The first evidence that BIO can directly induce cancer cell death came with the finding that BIO treatment produced apoptosis in human leukemia cells by down-regulating the anti-apoptosis factor, survivin (Holmes et al. 2008). Further indications that BIO may be useful as an anti-cancer agent came from studies of the enzyme, telomerase, which maintains telomere length and is linked to cell immortalization (Bilsland et al. 2009). Cancer cells overexpress telomerase, and it was observed that treatment with BIO for 5 weeks decreased telomerase reporter expression in human carcinoma cells. Significantly, human ovarian carcinoma cells xenografted into immunocompromised athymic mice showed reduced tumor formation after intraperitoneal BIO treatment, along with inhibited telomerase activity in the tumor cells (Bilsland et al. 2009). Cell signaling pathway analysis was used to link the bioactivity of BIO with telomerase inhibition. This pathway analysis illustrated the multiple effects of BIO treatment on cancer cell physiology (Fig. 3). This study also provided the first validation that BIO could be an effective anti-cancer agent in vivo and was confirmed in a mouse model of acute myeloid leukemia (AML), in which BIO treatment induced AML cell apoptosis and prevented host engraftment (Song et al. 2010).

Although BIO was initially characterized as a specific inhibitor of GSK-3β, further research revealed that this compound has activity against the Janus activated kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling protein (Liu et al. 2011). In cancer cells, the JAK/STAT3 pathway is activated and has been shown to contribute to carcinogenesis, providing a promising drug target (Ghoreschi et al. 2009). In this study, inhibition of JAK/STAT3 induced apoptosis via down-regulation of the anti-apoptosis factor, Mc-1. The in vivo effect of BIO against tumorigenesis in melanoma cells was confirmed in a mouse xenograft model. Of note, BIO was effective in this study using oral delivery (50 mg/kg daily).

Metastasis is the main cause of cancer mortality (Vatandoust et al. 2015). Thus, drugs that can block the metastatic spread of cancer cells would have a major impact on cancer patient survival. Employing the 4T1

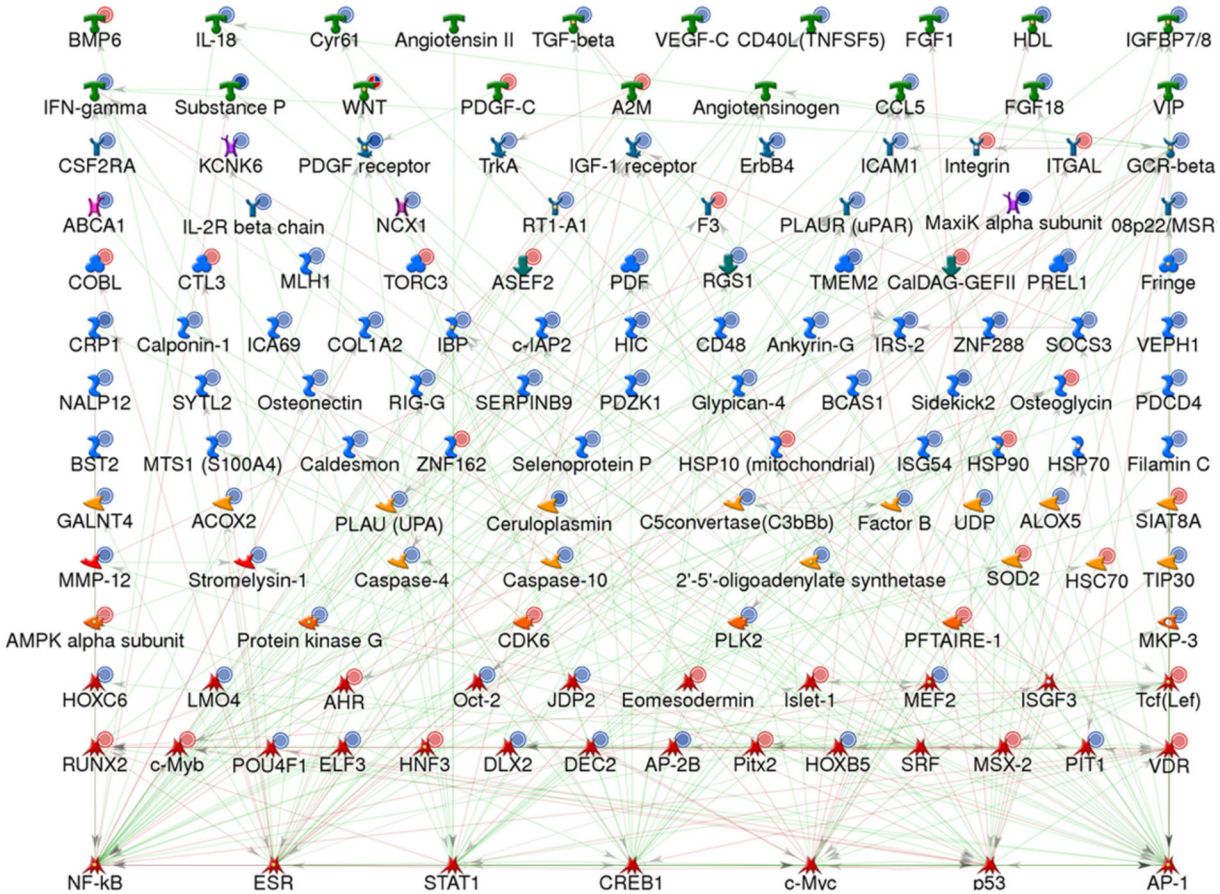


Fig. 3 An example of the pleiotropic effects of small molecule treatment in cancer cells. Genes showing differential expression in carcinoma cells treated with the cell reprogramming small molecule, BIO, for 21 days were identified using a whole genome expression array. *Blue circles* down-regulated in BIO treated cells; *red circles* up-regulated. *Shading intensity* indicates the fold-change of gene expression (minimum fivefold). *Green arrows* show pathway activation; *red arrows* show pathway inhibition. Image reproduced from PLoS One. Jul 31;4(7):e6459. doi: 10.1371/journal.pone.0006459, under the Creative Commons Attribution (CC BY) license

mouse model of aggressive breast cancer, it was observed that 1 mg/kg BIO pre-treatment dramatically reduced lung metastasis 8 days after intravenous delivery of cancer cells (Braig et al. 2013). BIO treatment reduced the cell invasiveness by down-regulating expression of the pro-migratory factors, C-terminal tensin-like protein, and matrix metalloproteinase 2, which was also linked to inhibition of the JAK/STAT3 pathway. Using RNAi-mediated reduction of target gene expression, it shown that BIO inhibited the signaling molecule 3-phosphoinositide-dependent protein kinase-1 (PDK1), in addition to JAK/STAT3 and GSK-3β, to produce this anti-metastatic effect. Thus, BIO can be considered as an interesting example of "polypharmacology" (drugs that affect multiple targets or disease-related pathways) and illustrates one of the advantages of developing pleiotropic small molecules as pharmaceutical candidates.

This concept of BIO as a polypharmacology agent for anti-cancer therapy was reiterated in a recent study of drug resistance in cancer cells. Tumor necrosis factor (TNF) α-related apoptosis inducing ligand (TRAIL) is an inducer of cell death that has been shown to induce apoptosis in a variety of different cancer cell types without affecting normal cells (Fesik 2005). However, many tumors, such as breast and bladder cancer, develop resistance to TRAIL-induced death, which is linked to survival mechanisms, such as the BIO target, GSK-3β (Koschny et al. 2007). Using concentrations of BIO

that are not cytotoxic for breast and bladder cancer cells, it was shown that the TRAIL pathway became reactivated and the cells were sensitized to TRAIL-induced apoptosis (Braig et al. 2014). Thus, BIO could be employed in combined therapy with TRAIL-inducing agents, such as SuperKillerTRAIL, to overcome chemoresistance in refractory tumors.

In summary, the development of the GSK-3β inhibitor BIO as an anti-cancer agent has led to the discovery that this compound possesses additional biological activity against the JAK/STAT3 pathway, which is a major regulator of carcinogenesis. Consequently, BIO is the subject of a number of patents related to anti-cancer applications (Gaboriaud-Kolar et al. 2015). A list of the reported anti-cancer activities of BIO is shown in Box 2. In the final part of this commentary, we recommend a quick and simple animal model system that can be used to facilitate bioactive compound repositioning as anti-cancer agents.

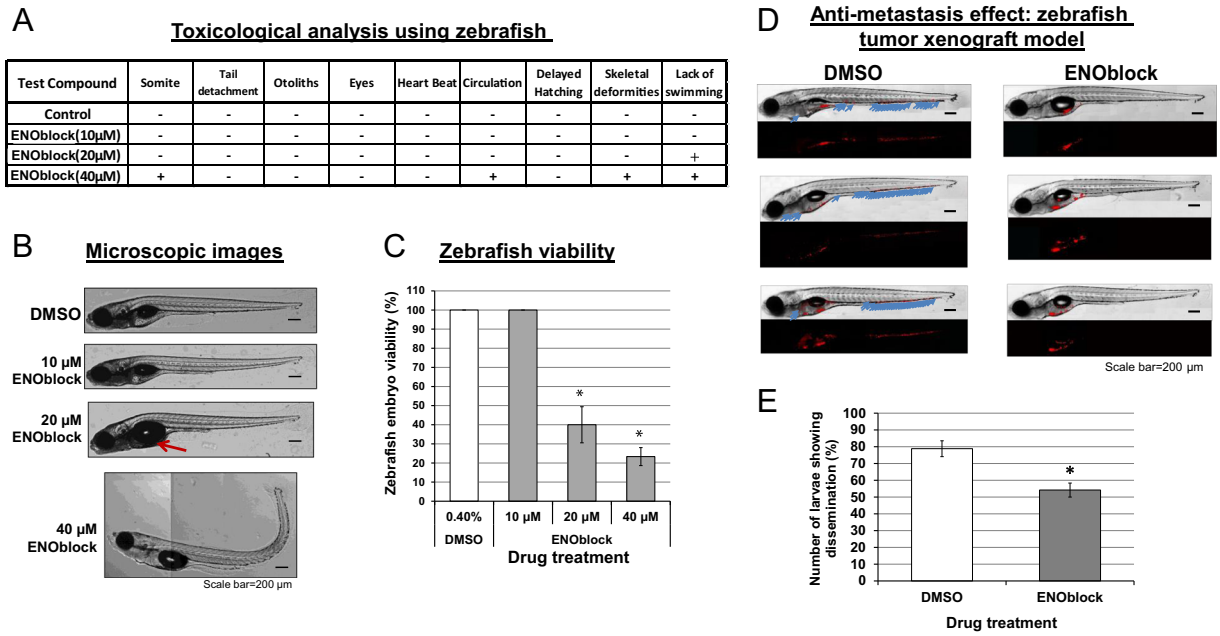
Box 2: Summary of current knowledge about the use of BIO in anti-cancer research

(1) Reduction of melanoma cell proliferation and migration, without affecting invasion, chemotoxicity, or apoptosis (Chon et al.)
(2) Induction of human melanoma cell apoptosis by functioning as a pan-JAK inhibitor selectively inhibiting STAT3 signaling (Liu et al.)
(3) Reduction of migration and promotion of the cytoskeletal rearrangement of stress fibers and focal adhesions in pediatric glioma (Cockle et al.)
(4) Suppression of ovarian cancer cell development via up-regulation of p21 expression (Yu and Zhao)
(5) Simultaneous inhibition of JAK/STAT3, PDK1, and GSK-3β to induce anti-metastatic activity in vivo (Braig et al.)
(6) Up-regulation of p21 to induce G2/M cell cycle arrest and activate caspase-dependent and caspase-independent apoptosis in invasive breast cancer cells (Nicolaou et al.)
(7) Reduction of pro-tumorigenic telomerase activity via modulation of multiple gene regulatory networks in a mathematical model; validated in vivo (Bilsland et al.)
(8) Reduction of osteolytic regions in multiple myeloma via targeting osteogenesis in bone marrow mesenchymal stem cells (Gunn et al.)
(9) Augmentation of TRAIL-induced apoptosis in various cancer cell lines (Braig et al.)
(10) Preservation of hematopoietic stem cell activity and inhibition of leukemic cell growth via down-regulation of survivin (Holmes et al. 2008)
(11) Suppression of cell growth and induction of apoptosis in human leukemia cell lines of diverse origin via modulation of the cell death regulator, Bcl-2 (Song et al. 2010)
(12) Modulation of *MYCN* expression to inhibit neuroblastoma cell viability via multiple pathways (Duffy et al. 2014)

The zebrafish model: swimming into view as a simple and powerful method to validate anti-cancer activity in vivo

As mentioned above, the cell reprogramming compounds, BIO and reversine, have both been shown to possess anti-cancer activity that was validated in animal models. In both cases, this was reported some years after initial characterization of the compound. However, rapid and experimentally convenient animal models have been established for anti-cancer analysis, which could allow "in-house" testing for repositioning, rapid publication, and patenting. An attractive model is the zebrafish cancer xenograft system, which has multiple advantages, such as simple housing requirements, rapid development, transparency for microscopic analysis of organ systems, and high genetic homology to humans compared with other non-mammalian models (approximately 80 % for zebrafish, compared to ≈60 % for the fruit fly, *Drosophila melanogaster*, and ≈36 % for the roundworm, *Caenorhabditis elegans*) (Mackay and Anholt 2006; Barbazuk et al. 2000; C.elegans 1998). These features have allowed the zebrafish to be used for studying pivotal aspects of carcinogenesis and metastasis (Ignatius et al. 2012; Blackburn et al. 2014). For example, zebrafish, mice, and humans develop tumors that show histological and genetic similarities. *APC* mutant tumors from these three species all form in the liver and intestine and show constitutive activation of Wnt signaling (Haramis et al. 2006).

The human xenograft system in zebrafish is established as a valuable tool for anti-cancer drug discovery (for example, (Jung et al. 2012; Trede et al. 2013; Jung et al. 2014a; Tulotta et al. 2016)) and can be set up using only a few fish tanks and a microinjector (Tabassum et al. 2015). In our own laboratory, this zebrafish system has been utilized for the rapid assessment of anti-cancer activity in novel compounds, along with toxicological analysis that can be used to predict potential teratogenic effects in mammals (Sipes et al. 2011; Jung et al. 2014a; Avallone et al. 2015). An example is provided in Fig. 4. Therefore, compounds that modulate targets linked to carcinogenesis can be conveniently tested for anti-cancer activity using this zebrafish xenograft system. This can both facilitate compound repositioning as anti-cancer agents and alleviate a major bottleneck in the drug development process: the failure of candidate compounds to be effective in animal systems (Chakraborty et al. 2009).



Test Compound	Somite	Tail detachment	Otoliths	Eyes	Heart Beat	Circulation	Delayed Hatching	Skeletal deformities	Lack of swimming
Control	-	-	-	-	-	-	-	-	-
ENOblock(10μM)	-	-	-	-	-	-	-	-	-
ENOblock(20μM)	-	-	-	-	-	-	-	-	+
ENOblock(40μM)	+	-	-	-	-	+	-	+	+

Fig. 4 An example of the use of the zebrafish model to determine toxicology and in vivo anti-cancer activity. **a**, **b** A novel bioactive compound, ENOblock (Jung et al. 2014b), was shown to be tolerated by developing zebrafish larvae up to a dose of 10 μM for 72 h. A panel of developmental markers are assessed to determine compound toxicity. The *red arrow* indicates deformities in the swim bladder at 20 μM dose. A dose of 40 μM ENOblock produced multiple abnormalities in the larvae. **c** Measurement of larvae viability after 72 h treatment with compound. **d**, **e** The human tumor xenograft model can measure cancer cell metastatic behavior in the zebrafish larvae. In untreated larvae, DiI labeled human colon carcinoma cells (*red* fluorescence) injected into the yolk sac have migrated to distal fish tissues (indicated using *blue arrows*). Larvae treated with 10 μM ENOblock for 96 h are viable, and the human cancer cells cannot invade into surrounding tissues; they are retained at the yolk sac injection site. *$p<0.05$ compared to DMSO treated larvae. (Figure adapted with permission from (Jung et al. 2013) ACS Chem Biol. 2013;8(6):1271-82. Copyright (2016) American Chemical Society)

Summary and future perspectives

In the commentary, we have discussed the link between stem cell reprogramming small molecules and their potential repositioning as anti-cancer compounds. Two examples were discussed: the compounds BIO and reversine, which were initially characterized as stem cell reprogramming agents and have been subsequently repositioned and patented as anti-cancer agents. We also present the zebrafish human tumor xenograft model as a rapid validation system for testing anti-cancer candidate compounds. The use of small molecules to control biological systems is increasing in scope, and this is especially relevant for the stem cell biology field. Very recently, novel small molecule cocktails have been developed that allow the generation of chemically iPSCs (ciPSCs), which are produced solely by small molecule treatment (Hou et al. 2013; Zhao et al. 2015; Ye et al. 2016). Novel small molecules and optimized small molecule-based methodologies for somatic cell reprogramming into stem cells are in continuous development and now aim to directly modulate patient cells in vivo (reviewed in (Anwar et al. 2016; Davies et al. 2015)). Many of these small molecules modulate cellular targets that are also linked to carcinogenesis, such as aurora kinases, GSK-3β, JAK/STAT3, and histone deacetylases.

It is now established that cancer cells possess many similar properties to normal stem cells (Hong et al. 2015). Therefore, it may seem counterintuitive that small molecules modulating stem cell phenotype can also possess anti-cancer activity. However, these anti-cancer effects can be explained by the pleiotropic nature of bioactive small molecules and the importance of cell context. In the case of the aurora kinase inhibitor, reversine, communication between cancer cells and stromal fibroblasts is disrupted, leading to reduced stromalization in developing tumors. BIO was initially characterized as a GSK-3β inhibitor, but the anti-cancer activity of BIO was discovered to be additionally related

to the inhibition of Jak/STAT3 signaling. Thus, the cellular and disease scenario are pivotal influences on small molecule activity and their potential repositioning. Given the current high priority placed on compound repositioning in the drug development and discovery process, especially in cancer therapeutics (Wurth et al. 2016), this may encourage researchers developing small molecules in stem cell research to also consider the possible value of their compounds as anti-cancer agents. Repositioning within the same research institution may also simplify any intellectual property issues surrounding the original compound.

Currently, the drug discovery pipeline is contracting (Bailey et al. 2014; Dean et al. 2014; Spellberg et al. 2015). Strategies that facilitate the repositioning and development of novel small molecule therapeutics are attractive strategies to tackle this problem because other technologies, such as gene therapy and cell therapy, have not yet realized their full potential (Hulot et al. 2016; Lysy et al. 2016). In this commentary, we have discussed the investigation of anti-cancer activity in cell reprogramming compounds, based on the "traditional" laboratory approach of cell-based assays and animal model testing. However, there are alternative strategies to facilitate repositioning, such as in silico-based methodologies utilizing large-scale virtual screening of compound libraries or compounds approved for human use against large numbers of protein targets (high-throughput shotgun repurposing) (Wang et al. 2013). We hope that this commentary illustrates the link between some cell reprogramming compounds and potential anti-cancer activity, based on the modulation of target proteins that are important regulators in both cell reprogramming and carcinogenesis.

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