Cancer Science
Japanese Cancer Association JCA
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Review Article

Safely targeting cancer stem cells via selective catenin coactivator antagonism

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Key words

Asymmetric, CREB-binding protein, p300, symmetric, Wnt/catenin

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Funding information

Support from the USC Norris Comprehensive Cancer Center (Support Grant P30 CA014089) and the US National Institutes of Health (NIH) grants 1R01CA166161-01A1, 1R21NS074392-01, 1R21AI105057-01 and NIH 1R01 HL112638-01 are gratefully acknowledged.

Received June 2, 2014; Accepted June 24, 2014

Cancer Sci **105** (2014) 1087–1092

doi: 10.1111/cas.12471

Throughout our life, long-lived somatic stem cells (SSC) regenerate adult tissues both during homeostatic processes and repair after injury. The role of aberrant regulation of SSC has also recently gained prominence in the field of cancer research. Following malignant transformation, so termed cancer stem cells (CSC), endowed with the same properties as SSC (i.e. the ability to both self-renew and generate differentiated progenitors), play a major part in tumor initiation, therapy resistance and ultimately relapse. The same signaling pathways involved in regulating SSC maintenance are involved in the regulation of CSC. CSC exist in a wide array of tumor types, including leukemias, and brain, breast, prostate and colon tumors. Consequently, one of the key goals in cancer research over the past decade has been to develop therapeutic strategies to safely eliminate the CSC population without damaging the endogenous SSC population. A major hurdle to this goal lies in the identification of the key mechanisms that distinguish CSC from the normal endogenous tissue stem cells. This review will discuss the discovery of the specific CBP/catenin antagonist ICG-001 and the ongoing clinical development of the second generation CBP/catenin antagonist PRI-724. Importantly, specific CBP/catenin antagonists appear to have the ability to safely eliminate CSC by taking advantage of an intrinsic differential preference in the way SSC and CSC divide.

Metastasis, multi-drug resistance and disease relapse constitute the central challenge for the successful treatment of advanced malignancies. Tumor initiation, metastasis and disease relapse have all recently been attributed to subpopulations of self-renewing, highly tumorigenic, drug-resistant tumor cells termed cancer stem cells (CSC) or, alternatively, tumor initiating cells (TIC).[1] CSC have many of the same attributes that their normal somatic stem cell (SSC) counterparts are endowed with, in that they have the ability to both self-renew and go on to more differentiated cell types. SSC, alternatively termed tissue stem cells, reside in specialized niches within tissues or organs (e.g. hematopoietic stem cells, neuronal stem cells and intestinal stem cells) and are critical during development as well as in the adult for both normal tissue homeostasis and regeneration after injury.[2] Therefore, a recent major focus in cancer research has been to develop therapeutic strategies to safely eliminate the CSC population without deleterious effects on the normal SSC population.

To safely accomplish this goal, we need to identify the key mechanisms that regulate stem cell self-renewal versus differentiation and, even more critically, the features that distinguish the control of self-renewal of CSC from their normal endogenous SSC counterparts. However, essentially the same evolutionarily conserved signaling pathways that govern embryonic development (i.e. Wnt/β-catenin,[3,4] Hedgehog[5] and Notch[6]) appear to control the behavior of both normal SSC as well as CSC. CSC express similar markers and exhibit cellular behaviors highly reminiscent of SSC. One conclusion that can be drawn from the literature is that there are multiple points of intersection and crosstalk, including feedback and feed-forward loops, connecting the various signaling cascades that modulate "stemness." The focus of this review will be on the role of nuclear catenin (both β-catenin and γ-catenin/plakoglobin) in the transcriptional regulation of CSC and the prospects for safely and effectively targeting catenin coactivator interactions to eliminate the CSC population in cancer.

Cancer Stem Cells and Their Role in Tumorigenesis

Cohnheim *et al.*[7] first proposed the concept that cancer might arise from a rare population of cells with stem cell-like properties almost 150 years ago. More recently, increasing evidence has confirmed the existence of a small subset of cells termed cancer stem cells (CSC) or, alternatively, tumor initiating cells (TIC), which are believed to be responsible for tumor initiation, drug resistance and metastasis. Acknowledgement of the presence of CSC has forced a paradigm shift from the earlier models of tumor homogeneity towards one of tumor hierarchy,

where CSC play the critical role.[8] The CSC concept postulates that a small population of CSC provide for the long-term maintenance of the tumor, whereas the bulk of the tumor consists of rapidly proliferating and differentiated (albeit aberrantly or only partially differentiated) cells. CSC are able to self-renew,[9] actively express telomerase[10] and express multidrug resistance pathways.[11,12] CSC are generally quiescent, but can give rise to rapidly dividing transient amplifying cells, which form the bulk of tumor cells (Fig. 1). Despite some still existing controversy regarding the CSC hypothesis,[13] it is clear that distinct cancer cell populations have enhanced tumorigenic capacity compared to bulk tumor cells. John Dick and colleagues first isolated CSC (known as leukemic stem cells [LSC]) from bulk acute myeloid leukemia cells in 1997.[14] LSC maintained or reacquired the ability to proliferate indefinitely without proper differentiation.[15] Over the past decade, a large number of studies have also identified CSC in solid tumors, including brain,[16] melanoma,[17] breast,[18] liver,[19] pancreatic[20] and colon cancer.[21]

Catenin Dependent Transcription and "Stemness"

The entry of catenin (classically β-catenin, although other catenins [.g. γ-catenin/plakoglobin] may also play a critical role)[22] into the nucleus and the subsequent transcriptional processes affected by β-catenin are classically controlled by the so termed "canonical Wnt" signaling cascade. However, there are a number of alternative signaling pathways that can induce the nuclear translocation of β-catenin and its subsequent participation in transcription. For example, the process of epithelial to mesenchymal transition (EMT) involves down-regulation of E-cadherin, which normally binds cytoplasmic β-catenin in a complex with α-catenin that stabilizes epithelial architecture,[23] leading to the subsequent nuclear translocation of β-catenin.[24] EMT is a hallmark of metastasis[25] and has also been implicated in the generation of CSC.[26] A variety of receptor tyrosine kinases[27] and non-receptor tyrosine kinases including Src[28] and Abl[29] can also disrupt the E-Cadherin /β-catenin interaction, thereby enhancing β-catenin mediated transcription. In addition, hypoxia[30,31] and high glucose levels[32] can also activate β-catenin mediated signaling. It is clear that a wide range of signaling molecules and cascades also influence β-catenin dynamics and β-catenin transcription.[33–35]

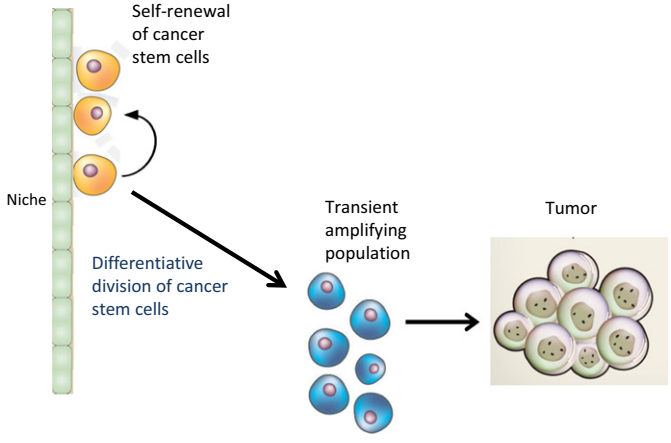

Fig. 1. Cancer stem cells both self-renew and undergo differentiative divisions to maintain or expand the cancer stem cell population or generate transient amplifying cells that go on to rapidly divide to form the bulk of the tumor.

In collaboration with signals from a number of other key pathways (e.g. Notch, Hedgehog, JAK/Stat, BMP, Hippo and FGF/MAPK), Wnt glycoproteins and, in particular, nuclear β-catenin, play essential roles in balancing self-renewal versus differentiation of adult stem cells (Fig. 2). However, there has been enormous controversy regarding whether Wnt signaling is important for proliferation and maintenance of potency (pluripotency or multipotency)[1,36,37] or differentiation of stem/progenitor cells.[38,39] Wnt/β-catenin signaling has been shown to maintain pluripotency in ES cells[37] and expand neural stem/progenitors, thereby increasing brain size.[40] However, Wnt/β-catenin signaling is also required for the differentiation of ES cells,[41] as well as fate determination in neural crest stem cells.[42] Clearly, Wnt/β-catenin signaling plays dichotomous roles in stem cell biology.

Wnt/Catenin, Cancer and Cancer Stem Cells

Wnt signaling is an enormously complex and ancient pathway that dates back to the first anaerobic metazoans. The Wnt/catenin pathway is critical in both normal embryonic development and throughout the life of the organism in virtually every tissue and organ system.

The pathway has emerged as a pivotal player in the specification and maintenance of stem cell lineages in multiple stem cell compartments in a wide array of tissues and organs, including intestines, the heart, and hematopoietic, neuronal and mammary glands.[43] Therefore, not surprisingly, a recurrent theme in cancer biology involves the aberrant regulation of Wnt signaling.[44,45] The discovery in 1991 that mutations in the tumor suppressor adenomatous polyposis coli (APC)[46,47] were associated with >80% of sporadic colorectal cancers via aberrant activation of Wnt signaling provided significant rationale to therapeutically target this pathway. APC is the most frequently mutated gene in human cancers.[48,49] However, mutations affecting the Wnt pathway are not restricted to colon cancer. Loss-of-function mutations in Axin have been found in hepatocellular carcinomas, and oncogenic β-catenin mutations, first described in colon cancer and melanoma,[50] have also been found in a wide variety of solid tumors,[51] including hepatocellular carcinomas,[52] thyroid tumors[53] and ovarian endometrioid adenocarcinomas.[54]

Safely Targeting Cancer Stem Cells

The significant role of aberrant Wnt signaling in cancer and CSC has engendered substantial efforts into the development of therapeutic approaches to target this pathway. However, a number of factors have thwarted progress in this field. First, the Wnt signaling cascade is bewilderingly complex, in that in mammals there are 19 Wnt ligands and more than 15 receptors and co-receptors distributed over seven protein families,[55] and this represents only the tip of the iceberg in regards to the difficulty in attempting to develop safe and effective specific Wnt pathway therapeutics. Further adding to the complexity of targeting transcriptionally competent β-catenin is the fact that β-catenin can bind to a broad spectrum of transcription factors other than TCF/LEF. Transcriptionally active β-catenin therefore modulates a plethora of downstream biological processes, including pluripotency, EMT, oxidative stress and lineage commitment.[56]

Successful therapeutic manipulation of endogenous "stemness" (normal or cancerous) will require significant precision to affect the desired transformations without deleterious effects

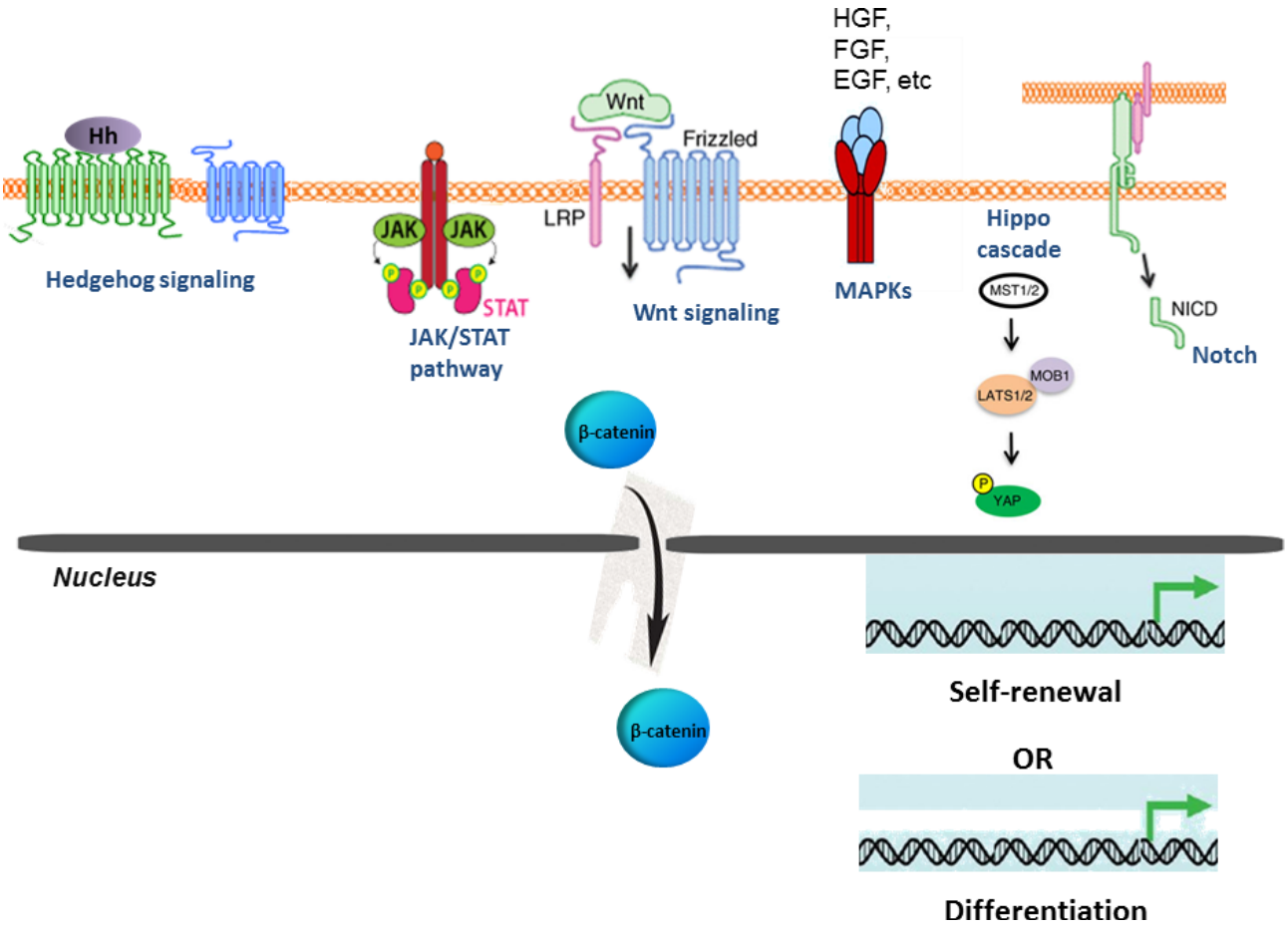

Fig. 2. The ultimate decision for a cell to retain potency or initiate differentiation is dependent upon numerous inputs from various signaling pathways (e.g. JAK/STAT, Wnt, Growth Factors, Hippo, Notch and Hedgehog) that also play critical roles during development. In the end, those multiple pathways must be integrated and funneled down into a simple decision point. β-catenin plays a central role in integrating these signals.

(depletion, in particular) to the normal stem cell populations.[4] Thus, the ability to target aberrant catenin transcriptional signaling offers enormous promise. However, just like the Sword of Damocles, significant risks and concerns regarding targeting such a critical pathway in stem cell maintenance and tissue homeostasis are ever present.

Differential Coactivator Modulation

To generate a transcriptionally active complex, β-catenin must recruit one of the two Kat3 transcriptional coactivators, cAMP response element binding protein (CREB)-binding protein (CBP) or its closely related homolog p300 (E1A-binding protein, 300 KDa), as well as other components of the basal transcriptional apparatus.[57–59] Recent studies have documented that CBP and p300 interact with hundreds of proteins in their roles as master orchestrators of transcription. Due to their high degree of homology, these two coactivators have long been considered as largely redundant. However, accumulating evidence indicates that CBP and p300 are not redundant but have definitive and unique roles both *in vitro* and *in vivo*.[60–62]

Using the TopFlash reporter gene system in SW480 colon carcinoma cells, we identified ICG-001 from a library of 5000 secondary structure mimetics. ICG-001 (Fig. 3) had an IC50 value of 3 μM in this assay. Using an affinity chromatography approach, we identified and subsequently validated that ICG-001 binds specifically and with high affinity (approximately 1 nM) to the coactivator CBP, but, importantly, not to its closely related homolog p300, despite the fact that these two coactivators are up to 93% identical, with even higher homology, at the amino acid level.[63,64] We demonstrated that selectively blocking the interaction between CBP and β-catenin with ICG-001 led to the initiation of a differentiation program in a wide variety of stem/progenitor cells.[65,66] This led us to develop our model of differential coactivator usage, which highlights the distinct roles of the coactivators CBP and p300 in catenin-mediated transcription.[58] The critical decision by β-catenin to utilize either CBP or p300 is the first decision that guides the cell to either proliferate/maintain potency or initiate a differentiation transcriptional program, respectively (Fig. 4).

Subsequently, we have identified several small molecules (IQ-1, ID-8 and, most recently, YH249/250) that selectively block the p300/β-catenin interaction, thereby increasing the CBP/β-catenin interaction, which maintains potency (pluripotency or multipotency) in a variety of stem cell populations, both in mouse and human.[65,67–69] The therapeutic potential of the selective CBP/β-catenin antagonist ICG-001 has been examined in a variety of preclinical tumor models, where it has demonstrated the ability to safely eliminate drug-resistant tumor-initiating cells.[70–72]

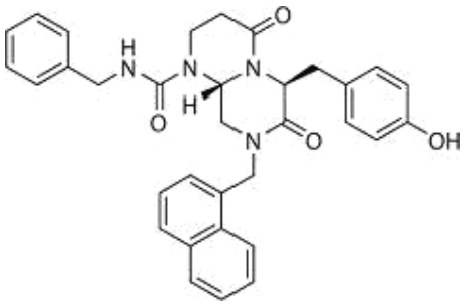

Fig. 3. Chemical structure of the CBP/catenin antagonist ICG-001.

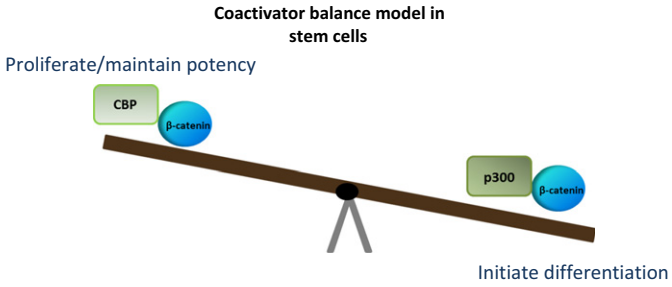

Fig. 4. Wnt signaling is a complex pathway, believed to be involved in the regulation of divergent processes, including the maintenance of pluripotency and commitment to differentiation. We developed a model in which β-catenin/CBP-mediated transcription is critical for the maintenance of potency, whereas β-catenin/p300-mediated transcription is the first critical step to initiate differentiation. Hence, the balance between CBP and p300-mediated β-catenin transcription regulates the balance between maintenance of potency, and the initiation of commitment to differentiate in stem and progenitor cells.

Interestingly, CBP/β-catenin antagonists have also demonstrated efficacy in a variety of injury models, including pulmonary and renal fibrosis[73,74] and myocardial infarction.[75] It appears that the differential effects of CBP/β-catenin antagonists on CSC versus normal SSC (i.e. forced differentiation and elimination versus differentiation and enhanced repair without apparent depletion) are apparently cell intrinsic and not due to the selective targeting by CBP/β-catenin antagonists of CSC versus normal SSC. We proposed that CBP/β-catenin antagonists take advantage of the intrinsic propensity of CSC to increase their number of symmetric divisions at the expense of asymmetric divisions due to various mutations (e.g. p53 and PTEN).[76,77] Normal endogenous long-term repopulating stem cells preferentially divide asymmetrically with one daughter cell remaining in the niche and the other going on to a transient amplifying cell required for generating the new tissue involved in repair processes.[78] However, if CSC undergo more symmetric differentiative divisions when treated with CBP/catenin antagonists, the CSC in the niche will eventually be cleared out, whereas normal SSC that divide asymmetrically will always maintain one of the dividing daughter cells in the stem cell niche (Fig. 5). This fundamental and cell intrinsic difference between SSC and CSC provides a unique opportunity to therapeutically target CSC without damaging the normal endogenous stem cell populations utilizing specific CBP/catenin antagonists.[78]

To The Clinic

Although the Wnt signaling pathway was discovered over 30 years ago, only recently have therapeutic agents that specifically target the Wnt pathway been introduced into clinical trials, although a few US Food and Drug Administration (FDA)-approved drugs do affect Wnt signaling, albeit non-specifically.[4] Despite intensive investigation of the pathway and the unveiling of a multitude of potential therapeutic points of intervention in the pathway, as well as the identification of reagents that interfere with some of these targets, it is still unclear whether most approaches will provide both clinical efficacy and safety. To date, pre-clinical and clinical experience with both small molecules and biologics that target different

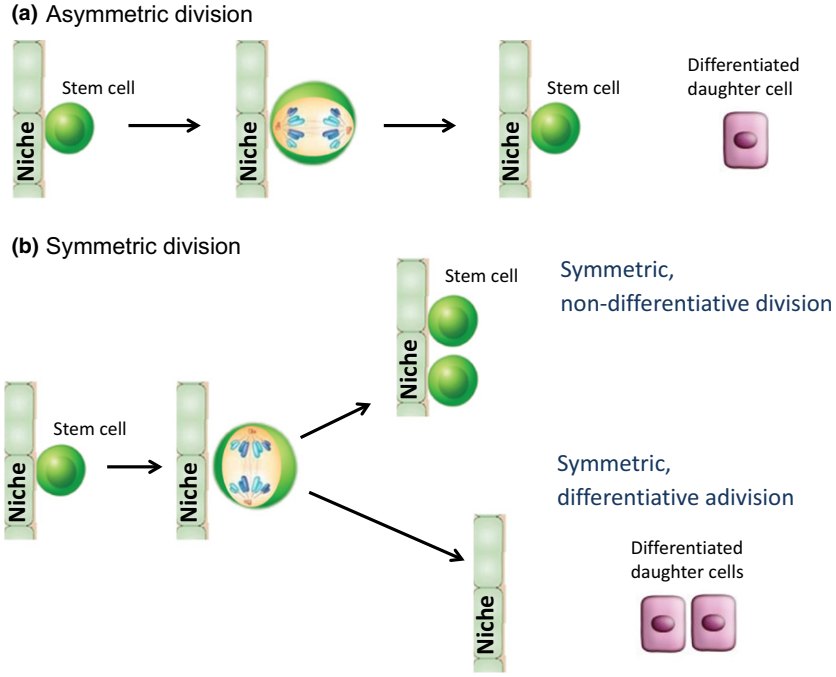

Fig. 5. Model depicting symmetric and asymmetric modes of division. The intrinsic difference between normal somatic stem cells (SSC) and cancer stem cells (CSC) is that normal SSC favor asymmetric division whereas CSC favor symmetric divisions. Treatment of CSC with CBP/catenin antagonists causes CSC to undergo symmetric differentiative divisions, thereby eventually clearing CSC from the niche. In sharp contrast, SSC undergo asymmetric divisions when treated with CBP/catenin antagonists.

points of intervention (porcupine, tankyrase, Fzd receptors and extracellular Wnt ligands) suggest that a therapeutic window does exist for the use of Wnt inhibitors in cancer patients. However, the full anti-tumor potential of these agents may not be realized due to side effects involving on target inhibition of Wnt/β-catenin signaling including intestinal toxicity and bone breakage.[79]

PRI-724, a specific CBP/catenin clinical compound. In principle, significant concerns about specificity could be raised about the use of small molecule inhibitors that target the coactivator protein CBP, which has perhaps as many as 500 molecular partners, including a wide array of transcription factors. However, to date, these concerns have not been borne out either preclinically or clinically. This is perhaps at first surprising and a full discussion of why a small molecule therapeutic that selectively targets the N-terminus of CBP has many therapeutic advantages is beyond the scope of this review.[78] However, a few salient features are worth mentioning: (i) the extremely high biochemical selectivity of ICG-001/PRI-724 for its molecular target; (ii) the disruption of only a small subset of CBP interactions; and (iii) the unique properties of the two Kat3 coactivators, CBP and p300.

PRI-724 is a second generation specific CBP/catenin antagonist (IC_{50}–150 nM) developed by Prism Pharma and partnered with Eisai Pharmaceuticals for oncology. PRI-724 proved to be extremely safe in pre-clinical investigational new drug enabling toxicology studies. The No Adverse Event Level for PRI-724 was 120 mg/kg/day in dogs given 28-day continuous infusion. An open label Phase Ia safety study in subjects with solid tumors, where the expression of the biomarker survivin /BIRC5 was measured by immuno-magnetic RT-PCR in circulating tumor cells for PRI-724 was initiated at USC in March 2011. The results of this trial were reported at ASCO in June 2013. In all, 18 patients were treated (dose escalation from 40 to 1280 mg/m^2/day) via continuous infusion for 7 days. Just as had been observed in preclinical studies, PRI-724 had a very acceptable toxicity profile, with only one DLT of grade 3 of reversible hyperbilirubinemia. Downregulation of the biomarker survivin/BIRC5 with upregulation of the differentiation antigen CK20 in circulating tumor cells strongly correlated with increasing plasma concentrations of the drug.[80] Additional trials with PRI-724 are underway, including combination trials with a modified Folfox6 regimen for refractory colorectal cancer patients, a Phase Ib trial for refractory pancreatic cancer patients in combination with gemcitabine and a Phase 1/2 trial for heme malignancies.

As mentioned above, CBP/β-catenin antagonists have also demonstrated efficacy in a wide variety of injury models, including pulmonary and renal fibrosis[73,74] and myocardial infarction.[75] Given the apparent safety of these agents both pre-clinically and clinically, additional clinical trials targeting these indications are anticipated in the future.

Acknowledgments

We would like to thank Dr Jia-Ling Teo for a critical review of and assistance with the preparation of this manuscript.

Disclosure Statement

The corresponding author is a consultant and equity holder in Prism Pharma Co. Ltd.

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