17

The Rocky Road from Cancer Stem Cell Discovery to Diagnostic Applicability

Paola Marcato and Patrick W. K. Lee
Dalhousie University
Canada

1. Introduction

Since the discovery of the discretely isolatable highly tumorigenic tumor population with self renewal and differentiation properties (i.e. cancer stem cells, CSCs), it has been theorized that their quantification in tumor tissues would have significant prognostic value. Based on their increased tumorigenicity and stem cell like qualities, it is postulated that patients with elevated levels of CSCs would more likely suffer from an aggressive form of disease that is comparatively resistant to currently employed therapeutics. The results of the studies evaluating the prognostic value of CSC markers have been mixed, and cancer and marker dependant. Herein we review the currently known CSC markers for the more common cancers and their respective prognostic potential. In conclusion we will discuss what this data potentially reveals about the role of CSCs in tumor progression.

2. Leukemia

2.a Identified leukemia stem cell markers

Acute myeloid leukemia (AML) is the result of malignant transformation of hematopoietic progenitor cells. These altered cells proliferate and lead to the accumulation of AML blasts. Only a minority population of all AML cells are capable of proliferating *in vitro* and *in vivo*. This suggests that AML cells are potentially organized in a hierarchy, with only the most primitive of these cells capable of maintaining the leukemic clone. This hypothesis was the basis for identifying the AML initiating cell and the CSC theory that arose from the subsequent studies. As such, CSCs were first identified in AML in 1990's by John Dick's group in Toronto, Canada (Bonnet and Dick, 1997; Lapidot et al., 1994). AML cells that were CD34+CD38- possessed stem cell like characteristics and *de novo* leukemia repopulating properties in immunocompromised mice (Bonnet and Dick, 1997). While leukemia initiating cells (LSCs) are in the range of 0.00002 to 0.02% of all unsorted mononuclear blood cells, LSCs were in the range of 0.02 to 2% in the CD34+CD38- sorted cells of tested patient samples (sorting resulting in approximate 100-1000 fold enrichment of the CSC population). In further similarity to normal stem cells, CD34+CD38- expression is also a unique identifier for hematopoietic stem cells (HSCs). Since then, other leukemic stem cell (LSC) markers have been identified.

In 1997 and 1998, Blair et al. explored the use of other known HSC markers for the isolation of AML initiating cells and found that AML cells that are $CD34^{+}/HLA\text{-}DR^{-}/CD71^{-}$ and $Thy1^{-}$

(CD90) had the CSC phenotype (Blair et al., 1997; Blair et al., 1998). Later that same group added lack of expression of c-kit (CD117) to the list of potential AML CSC markers, as AML cells from patients that were $CD34^{+}c\text{-}kit^{-}$ were enriched for the CSC population (Blair and Sutherland, 2000). In 2000, Jordan et al., identified a cell surface molecule, interleukin-3 receptor alpha chain (CD123) as being uniquely expressed on AML CSCs, but not HSCs (Jordan et al., 2000), allowing for a potential method of distinction between LSCs and HSCs.
Other than the above described cell surface markers used for the isolation of CSCs, "functional markers" have been explored more recently. The functional marker strategy is based on stem cell characteristics, but does not rely on cell surface adhesion molecules for the viable isolation of a specific cell-subset. For example, Stemcell Technologies developed the aldefluor assay for the isolation of live hematopoietic stem cells based on increased expression of a cytoplasmic enzyme, aldehyde dehydrogenase (ALDH) isoform 1A1. ALDH1A1 is one of 19 ALDH isoforms expressed in humans, and it is a critical detoxifying enzyme responsible for oxidizing aldehydes to carboxylic acids (Marchitti et al., 2008). While, predominantly expressed in the epithelium of testis, brain, eye, liver, and kidney, ALDH1A1, is also found in high levels in hematopoietic and neural stem cells (Armstrong et al., 2004; Chute et al., 2006; Marchitti et al., 2008). ALDH1A1 is proposed to play a role in the differentiation of hematopoietic and neural stem cells via the oxidation of retinal to retinoic acid (Collins, 2008). Retinoic acid activates nuclear retinoic acid receptors (RARs) and RARs subsequently regulate the transcription of genes with RAREs (retinoic acid response elements). Furthermore, ALDH1A1 is known to metabolize and detoxify chemotherapeutics like cyclophosphamide (Magni et al., 1996), and is therefore thought to contribute to the innate chemotherapeutic resistance properties of hematopoietic stem cells. Using the aldefluor assay, Cheung et al., were the first to show that it was possible to isolate the LSCs based on the increased ALDH activity (Cheung et al., 2007). The researchers detected a population of $ALDH^{+}$ AML cells in 14 of 43 patient samples. In the remaining 29 samples an ALDH+ population was rare or unidentifiable. The $ALDH^{+}$ AML cells in most cases co-expressed $CD34^{+}$ (the previously identified marker) and engrafted significantly better than the $ALDH^{-}$ AML cells in immunocompromised mice. As discussed later, ALDH activity would become one of the few markers discovered that has applicability across a wide range of cancers. It can be said that ALDH activity is a universal CSC marker.

2.b LSC markers as prognostic indicators

Since the identification of a LSC population using cell surface markers it has been postulated that these markers could be used for diagnostic purposes, where patients with comparatively increased CSC numbers would theoretically suffer poorer outcomes. The results of the studies for leukemia are summarized in Table 1. Many of the cell surface markers had been evaluated separately for prognostic value prior to their discovery as potential CSC markers. For example as early as 1989, studies were being completed evaluating the prognostic value CD34 in AML (Borowitz et al., 1989; Campos et al., 1989). By 2000, a study by Kanda et al., summarized the results from the then 22 completed independent studies on the prognostic relevance of CD34 for AML (Kanda et al., 2000). The authors' review of the literature revealed a wide heterogeneity of results, with 12 studies concluding that increased CD34+ was associated with worse outcome, while the other 10 studies failed to show the relevance of CD34 expression in predicting patient outcome (summarized in Table 1) (Kanda et al., 2000). Kanda et al., concluded that given the wide variability of conclusions from the reports, CD34 expression could not be employed reliably as a prognostic marker (Kanda et al., 2000).

Similar disparity was seen for CD38 prognostic studies. For example, in 1993, Koehler et al. reported that CD38 expression failed to significantly correlate with the outcomes of 325 patients of childhood acute lymphoblastic leukemia (ALL) (Koehler et al., 1993). In 2000, Keyhani et al. evaluated the levels of CD38 expression in the blasts of 304 AML and 138 ALL patients (Keyhani et al., 2000). Patients with the higher percentages of $CD38^+$ cancer cells had the best outcomes, experiencing both longer times between remission and relapse and improved overall survival. Their results infer that patients with increased $CD38^-$ (LSC marker) cancer cells experienced the worse outcomes. However, lack of CD38 expression was only a significant independent risk factor for the AML patients, not ALL patients. In 2003, Repp et al. assessed the prognostic value of a panel of 33 different CD molecules for AML (Repp et al., 2003). Among the panel, expression of CD38 and CD34 was quantified singly in 783 patient samples. As the CSC theory would predict, patients with increased CD34 expression or decreased CD38 expression had poorer overall outcomes.

Other LSC markers have also been tested for their prognostic value individually. In a 1994 immunophenotyping AML prognostic study (Bradstock et al., 1994), LSC proposed markers CD34, c-kit (CD117) and HLA-DR (Blair et al., 1998; Blair and Sutherland, 2000) were among the panel of CD antigens tested. CD34, c-kit and HLA-DR expression failed to correlate with patient outcome (Bradstock et al., 1994). A more recent study concluded that increased c-kit (CD117) expression correlated with worse outcomes for AML patients (Advani et al., 2008). This result was in direct disagreement with the CSC theory since it is the lack of c-kit expression in combination with CD34 expression that was used to identify LSCs (Blair and Sutherland, 2000).

The above described studies suggest that the prognostic potential of LSC markers is not promising and clinically irrelevant. However, as discussed below, employed in combination, the prognostic potential of LSC markers becomes more apparent and the results therefore lend support to the CSC theory. In 2005, van Rhenen et al., quantified the frequency of $CD34^+CD38^-$ cancer cells in 92 AML patients at time of diagnosis and reported worse outcomes for patients with increased $CD34^+CD38^-$ cancer cells (van Rhenen A. et al., 2005). Patients with increased $CD34^+CD38^-$ cancer cell frequency (>3.5%) relapsed on average 5.6 months post remission, while patients with lower $CD34^+CD38^-$ cancer frequency relapsed on average 16 months post remission. The prognostic value of $CD34^+CD38^-$ has also been observed in other leukemias. Recently, Ebinger et al. quantified the frequency of $CD34^+CD38^-$ leukemia blasts in 42 childhood ALL cases (Ebinger et al., 2010). The researchers found that increased $CD34^+CD38^-$ cancer cells was associated with increased minimal residual disease and thus poorer prognosis for this leukemia sub-type as well. Although future studies will be required for confirmation, it appears that using the CSC markers in combination is more relevant as a prognostic tool than their application as singly applied markers.

Finally, with the more recent discovery that ALDH activity can be used as marker to isolate LSC (Cheung et al., 2007), ALDH activity is also being tested for prognostic value. Cheung et al. reported that increased ALDH activity in AML patient samples correlated significantly with the cytogenetic changes previously associated with unfavourable prognosis (Cheung et al., 2007). In 2009, Ran et al. compared the outcomes of 40 AML patients with higher percentages of ALDH+ cancer cells (>0.36%) to 28 patients with lower frequencies of ALDH+ cells ($\leq$0.36%) (Ran et al., 2009). Increased frequency of $ALDH^+$ cells correlated significantly with decreased survival probability. We await the results of future studies that will test the prognostic potential of ALDH activity combined with the LSC cell surface markers.

LSC marker	Patient sample size	Prognostic correlation			Publication
		worse outcome	no correlation	improved outcome	
$CD34^+$	145	X			(Campos et al., 1989)
$CD34^+$	75	X			(Borowitz et al., 1989)
$CD34^+$	96	X			(Geller et al., 1990)
$CD34^+$	27	X			(Guinot et al., 1991)
$CD34^+$	154	X			(Solary et al., 1992)
$CD34^+$	126	X			(Lee et al., 1992)
$CD34^+$	38	X			(Myint and Lucie, 1992)
$CD34^+$	150	X			(Campos et al., 1992)
$CD34^+$	70		X		(Selleri et al., 1992)
$CD34^+$	80		X		(Ciolli et al., 1993)
$CD34^+$	60	X			(Fagioli et al., 1993)
$CD34^+$	52	X			(te Boekhorst et al., 1993)
$CD34^+$	62		X		(Lamy et al., 1994)
$CD34^+$	168		X		(Bradstock et al., 1994)
$CD34^+$	481		X		(Sperling et al., 1995)
$CD34^+$	99		X		(Fruchart et al., 1996)
$CD34^+$	42		X		(Arslan et al., 1996)
$CD34^+$	517		X		(Porwit-MacDonald et al., 1996)
$CD34^+$	62	X			(Dalal et al., 1997)
$CD34^+$	141	X			(Raspadori et al., 1997)
$CD34^+$	37	X			(Kyoda et al., 1998)
$CD34^+$	783	X			(Repp et al., 2003)
$CD38^-$	325		X		(Koehler et al., 1993)
$CD38^-$	442	X			(Keyhani et al., 2000)
$CD38^-$	783	X			(Repp et al., 2003)
c-kit^-	168		X		(Bradstock et al., 1994)
c-kit^-	152			X	(Advani et al., 2008)
HLA-DR^-	96			X	(Geller et al., 1990)
HLA-DR^-	154			X	(Solary et al., 1992)
HLA-DR^-	168		X		(Bradstock et al., 1994)
$CD34^+CD38^-$	92	X			(van Rhenen A. et al., 2005)
$CD34^+CD38^-$	42	X			(Ebinger et al., 2010)
$ALDH^+$	65	X			(Ran et al., 2009)

Table 1. Summary of results from immunohistological prognostic studies of LSC markers

3. Breast cancer

3.a Identified breast CSC markers

Breast cancer was the first solid tumor identified to have a population of tumor cells with an inherent highly tumorigenic quality. These cells were termed tumor propagating cells at the time, and are now more commonly known as breast CSCs. In 2003, Al-Hajj et al. performed experiments akin to the leukemia studies discussed above (Bonnet and Dick, 1997; Lapidot et al., 1994), and isolated sub-tumor cell populations based on cell surface marker expression (Al-Hajj et al., 2003). The group showed that as few as 10^2 $CD24^{-/low}CD44^+$ breast tumor cells could re-capitulate the tumor with much of its original heterogeneity (Al-Hajj et al., 2003). The authors proposed that not all $CD24^{-/low}CD44^+$ cells were CSCs but that the breast $CD24^{-/low}CD44^+$ population was enriched for CSCs. It was hypothesized that if additional breast CSC markers were identified it may be possible to isolate and even more highly tumorigenic cells and initiate a tumor in xenograft from only one cell. This led to the pursuit of the identification of additional markers, both cell surface and functional.

Using the same functional marker approach previously employed for leukemia (Cheung et al., 2007), Ginestier *et al.* were the first to isolate CSCs from a solid tumor based on increased ALDH activity (Ginestier et al., 2007). The researchers showed that as few as 10^2 ALDH+ breast cancer cell isolated from patients could induce tumors in immunocompromised mice. Further, in a proof of principle experiment, the researchers isolated $CD24^{-/low}CD44^+ALDH^+$ breast cancer cells and were able to induce tumors in immunocompromised mice with as few as 20 injected cells. This experiment combining mulitple markers for the isolation of highly tumorigenic cells provided supportive evidence to the proposed hypothesis that identifying additional markers would lead to further enrichment of the CSC population.

In another more recent approach using a functional markers to identify novel breast CSCs, Pece et al. isolated the human normal mammary stem cells (hNMSCs) from mammary reduction surgeries by retention of a lipophillic fluorescent dye, PKH26 (Pece et al., 2010). PKH26 stains quiescent cells, allowing for the isolation of relatively non-dividing cells from a mixed population of proliferating cells (Lanzkron et al., 1999). From these isolated putative stem cells, Pece et al. identified a unique gene expression signature, the hNMSC signature, and applied it to published breast cancer gene expression data sets (Pece et al., 2010). This analysis revealed that many of the genes upregulated in normal mammary stem cells were also upregulated in higher grade, aggressive breast cancers. When the authors picked a few of these upregulated genes (i.e.CD49+, $DLL1^{high}$, $DNER^{high}$) and used them as cell surface markers, they were able to identify and isolate a sub-population of highly tumorigenic cancer initiating cells from breast tumors. As such, PKH26 stain retention and $CD49+DLL1^{high}DNER^{high}$ are the most recent breast CSC markers identified. Interestingly, CD49 is a previously known normal mammary stem cell marker, and DLL1 and DNER have been connected to normal stem cell function.

3.b Breast CSC markers as prognostic indicators

CSC quantification is a proposed prognostic indicator for breast cancer. Translating this to clinical application requires immunohistological methods for identification of CSCs in fixed tumor tissue and in this respect, the data is less convincing and is summarized in Table 2. First, for $CD24^{-/low}CD44^+$ the published studies have been mixed. In 2005, Abraham et al. were the first to publish a study on the prognostic applicability of the then newly identified breast CSC markers (Abraham et al., 2005). The authors double stained

an archived panel of 122 fixed breast cancer patient tumor samples for the prevalence of $CD24^{-/low}CD44^{+}$. They failed to find a correlation between increased abundance of these cells and tumor progression or worse outcome, but they did note a tendency towards the development of distant metastases (Abraham et al., 2005). Subsequently, in 2008, Honeth et al. stained a panel of 240 breast cancer patient samples for $CD24^{-/low}CD44^{+}$ cells and found an association between basal-like and BRCA1 hereditary breast cancer and the presence of $CD24^{-/low}CD44^{+}$ cells (Honeth et al., 2008). Also in 2008, Mylona et al. stained a panel of 155 fixed patient tumor samples and reported that the prevalence of $CD24^{-/low}CD44^{+}$ cells did not significantly correlate with worse prognosis. In fact, in disagreement with the CSC theory they found the opposite. Surprisingly, patient tumors with increased $CD24^{-/low}CD44^{+}$ cells tended to manifest increased disease-free survival (Mylona et al., 2008).

Cultured cell experiments indicate that $CD24^{-/low}CD44^{+}$ breast cancer cells are relatively more resistant to currently used therapeutics (Phillips et al., 2006). This suggests that prevalence of $CD24^{-/low}CD44^{+}$ cells in patient tumors is a potential measure of the susceptibility of breast cancer to certain therapeutics. If this hypothesis is true, then one would predict that post treatment, the percentage of these cells would increase as the overall bulk of the tumor is decreased. In a recent neoadjuvant immunohistological study of an archived panel of patient tumor samples before and after treatment, Aulmann et al. failed to show an increase in the frequency of $CD24^{-/low}CD44^{+}$ cells post treatment (Aulmann et al., 2010). In contrast, in a challenge to the theory CSC of the therapeutic resistance of these cells, the authors found that post treatment, the percentage of $CD24^{-/low}CD44^{+}$ tumor cells decreased relative to pretreatment (Aulmann et al., 2010). Further, the prevalence of these cells in a tumor did not correlate with the patient's response to treatment or eventual outcome (Aulmann et al., 2010). However, in agreement with results by Abraham et al. who noted that patient tumors with higher percentages of $CD24^{-/low}CD44^{+}$ tumor cells tended to develop distant metastases (Abraham et al., 2005), Aulmann et al. reported that patient tumors with higher percentages of $CD24^{-/low}CD44^{+}$ cells tended to develop bone metastases with greater frequency (Aulmann et al., 2010).

The results from the above described immunohistological studies evaluating the prevalence of $CD24^{-/low}CD44^{+}$ cells in breast tumors as a readout for predicting the relative aggressiveness of a breast cancer do not support their use as prognostic indicators. This is surprising considering that the prevalence of $CD44^{+}$ cells alone in fixed breast tumor cells was discovered to be predictive of more aggressive disease long before CD44 was identified as CSC marker (Al-Hajj et al., 2003). CD44 is a recognized predictor of breast cancer tumor grade (a histoclinical assessment of tumor cells and accepted clinical prognostic indicator (Dalton et al., 2000)), where patients with tumor cells expressing higher levels of CD44 membrane proteins have worse outcomes (Joensuu et al., 1993; Looi et al., 2006; McSherry et al., 2007). In light of the undisputed correlation between $CD44^{+}$ tumors and worse outcome for breast cancer patients, it seems that at least employing $CD44^{+}$ as a CSC marker agrees with the proposed role of CSC in mediating cancer progression. Where the hypothesis fails is in the inclusion of CD24 as a CSC marker. Perhaps the inclusion of $CD24^{-/low}$ as a criterion is not necessary and may be detrimental, at least from a diagnostic perspective. In fact, even prior to its use as a selection criterion for breast CSC isolation, increased (not decreased!) CD24 expression had been correlated with worse outcome for breast cancer patients (Athanassiadou et al., 2009; Kristiansen et al., 2003).

With the revelation that breast CSCs could also be identified by increased ALDH activity, expression of ALDH1A1 prevalence in breast cancer tumors was assessed for prognostic applicability (Ginestier et al., 2007). In this first analysis, ALDH1A1 expression was detected in only 30% of fixed breast cancer tumor samples (Ginestier et al., 2007). Immunohistological staining of 577 fixed tumor specimens revealed a significant correlation between ALDH1A1 expression and higher tumor grade. While these patients also had worse outcome overall, ALDH1A1 positivity failed to correlate with cancer stage and metastasis at the time of diagnosis (Ginestier et al., 2007). Later, in contrast, for a rare highly aggressive form of breast cancer, inflammatory breast cancer (one to five percent of all breast cancers), Charafe-Jauffret et al. found a significant correlation between ALDH1A1 expression and development of metastasis and worse outcome (Charafe-Jauffret et al., 2010). However, despite this positive correlation with a rare breast cancer, others have failed to show a significant correlation with ALDH1A1 prevalence and higher tumor grade, metastasis, therapeutic resistance or outcome with breast cancer in general (Morimoto et al., 2009; Neumeister et al., 2010; Neumeister and Rimm, 2009; Resetkova et al., 2009). In 2009, Morimoto et al. double immunohistochemical stained a panel of 203 fixed breast cancer tumor sample for the prevalence of ALDH1A1 along with estrogen receptor (ER), Ki67 and HER2 receptor status (Morimoto et al., 2009). The authors failed to find a correlation between ALDH1A1 prevalence and metastasis, but did note a non-significant trend with higher grade tumors. As well, $ALDH1A1^{+}$ tumors were more likely to be ER^{-}, $Ki67^{-}$, and $HER2^{+}$ (Morimoto et al., 2009). Also in 2009, Resetkova et al. immunostained four panels of fixed breast cancer patient panels, an adjuvantly treated series of 245 samples, a neoadjuvantly treated series of 34 samples and two series of 58 and 44 ER-PR-HER2- carcinoma samples. ALDH1A1 expression correlated significantly with basal-like $HER2^{+}$ cancers, but not with other important indicators like metastasis. Interestingly, this result for ALDH1A1 was similar to the study on $CD24^{-/low}CD44^{+}$ prevalence published by Honeth et al. who described a similar correlation between basal like breast cancers and $CD24^{-/low}CD44^{+}$ abundance. This would suggest that there is an overlap between $ALDH1A1^{+}$ and $CD24^{-/low}CD44^{+}$ cells and supports the notion that both markers identify at least some of the same cell population (i.e. CSCs). The neoadjuvantly stained data set failed to show an enrichment of $ALDH1A1^{+}$ cells post treatment, therefore not supporting the hypothesis that CSC population is resistant to currently employed therapeutics (Resetkova et al., 2009). Interestingly, however, the authors noted an increased expression of $ALDH1A1^{+}$ in the stromal tissue post treatment, but overall higher expression in the stroma was associated with better outcomes. Most recently, Neumeister et al stained a panel of 639 breast cancer for ALDH1A1, CD44 and cytokeratin (Neumeister et al., 2010). While the prevalence of all three markers together was associated with worse outcome, staining the cohort of samples for ALDH1A1 alone failed to correlate with any of the prognostic indicators (e.g. tumor grade, lymph node metastasis), nor patient outcome (Neumeister et al., 2010). Overall, the published data does not lend strong support toward the prognostic potential of ALDH1A1 or $CD24^{-/low}CD44^{+}$. This has led to the suggestion that other breast CSC marker need to be identified, and has resulted in some scepticism as to the validity of the existing identified markers (Neumeister and Rimm, 2009). However, it is noted that when employed in combination, CD44 and ALDH1A1 prevalence did predict outcome for breast cancer patients (Neumeister et al., 2010), suggesting that the key may be using the CSC markers in combination.

Breast CSC marker	Patient sample size	Prognostic correlation			Publication
		worse outcome	no correlation	improved outcome	
$CD24^{-/low}$	201			X	(Kristiansen et al., 2003)
$CD24^{-/low}$	70			X	(Athanassiadou et al., 2009)
$CD44^{+}$	198	X			(Joensuu et al., 1993)
$CD44^{+}$	70	X			(Ozer et al., 1997)
$CD44^{+}$	152	X			(Bankfalvi et al., 1998)
$CD44^{+}$	135	X			(Schneider et al., 1999)
$CD44^{+}$	60	X			(Looi et al., 2006)
$CD24^{-/low}CD44^{+}$	122		X		(Abraham et al., 2005)
$CD24^{-/low}CD44^{+}$	240		X		(Honeth et al., 2008)
$CD24^{-/low}CD44^{+}$	155			X	(Mylona et al., 2008)
$CD24^{-/low}CD44^{+}$	50		X		(Aulmann et al., 2010)
$ALDH1A1^{+}$	577	X			(Ginestier et al., 2007)
$ALDH1A1^{+}$	203		X		(Morimoto et al., 2009)
$ALDH1A1^{+}$	381		X		(Resetkova et al., 2009)
$ALDH1A1^{+}$	109	X			(Charafe-Jauffret et al., 2010)
$ALDH1A1^{+}$	639		X		(Neumeister et al., 2010)
$ALDH1A1^{+}CD44^{+}$cytokeratin^{+}	639	X			(Neumeister et al., 2010)

Table 2. Summary of results from immunohistological prognostic studies of breast CSC markers

4. Brain cancer

4.a Identified brain CSC markers

Soon after breast CSCs were identified based on $CD24^{-/low}CD44^{+}$ expression, similar studies conducted by Sing et al. identified a sub-tumor population of glioblastoma (most common brain cancer) cancer cells that were highly tumorigenic. As few as 10^2 glioblastoma cancer cells expressiong neural stem cell marker $CD133^{+}$ (prominin 1) (Uchida et al., 2000) induced tumors in immunocompromised mice (Singh et al., 2003; Singh et al., 2004). In contrast to the $CD133^{+}$ brain tumor cells, the $CD133^{-}$ cells did not induce tumors, even when 10^5 cells were injected in the mice (Singh et al., 2004). As well, $CD133^{+}$ cells exhibited the self-renewal differentiation properties characteristic of CSCs (Singh et al., 2004). Interestingly, as discussed later, CD133 would become a prominent CSC marker used for the isolation of highly tumorigenic cells in a number of cancers. However, like in other cancers, additional markers have been explored for brain cancer as well.

Again taking cues from discoveries made from normal neural stem cell research, Ogdent et al. and Tchoghandjian et al. found that glioblastoma CSCs could be identified by increased expression A2B5 (Ogden et al., 2008; Tchoghandjian et al., 2010). A2B5 is a ganglioside expressed specifically on the cell surface of neural progenitor cells (Nunes et al., 2003). Unexpectedly, $CD133^{+}$ and $A2B5^{+}$ potentially identify separate populations of brain tumor cells that do not necessarily overlap, in a patient dependant manner. This finding challenges the CSC theory, which predicts the existence of a cancer initiating tumor cell population that is identifiable based on a universally expressed combination of markers.

In 2007, Barraud et al. found that stage-specific embryonic antigen 4 (SSEA4), a known cell surface pluripotent human embryonic stem cell marker could also be used to enrich for the neural stem cells (Barraud et al., 2007). Subsequently, Son et al. found that the same marker could be used to isolate brain tumor cells with the CSC phenotype (increased tumorigenicity, self-renewal/differentiation properties) (Son et al., 2009). Almost all patient samples tested contained a $SSEA4^{+}$ population, in agreement with the CSC theory (Son et al., 2009).

As of yet ALDH activity has not been explored as a marker for the isolation of brain CSCs. Given its applicability in a number of cancers (as discussed above and below), it would be surprising to find that it is not a relevant brain CSC marker.

4b. Brain CSC markers as prognostic indicators

There have been a number of studies addressing prognostic applicability of the first brain/glioblastoma CSC marker identified, $CD133^{+}$ (summarized in Table 3). First, in 2008, Zeppernick et al. performed immunohistochemical analysis on 95 patient glioma samples of varied tumor grade and histology (Zeppernick et al., 2008). The authors report that $CD133^{+}$ prevalence and clustering was associated significantly with worse outcome and survival. Further, $CD133^{+}$ was a risk factor for tumor regrowth and metastasis, independent of tumor grade. Later that year, Beier et al., quantified a set of 36 high grade oligodendroglial tumors (less than 10% of all neural cancers) for their CD133 positivity (Beier et al., 2008). The authors reported that CD133 prevalence was a more accurate predictor of worse outcome for the patients than histological grading. In another 2008 study, Pallini et al. analysed a cohort of 44 glioblastoma patient tumor samples for prevalence of $CD133^{+}$ and $Ki67^{+}$ cells (Pallini et al., 2008). While CD133+ expression alone failed to predict patient oucome, coexpression of CD133/Ki67 was a highly significant independent prognostic risk factor

with prevalence of $CD133^+Ki67^+$ tumor cells being correlative with quickened disease progression and poor clinical outcome. In 2008, Zhang et al. stained a panel of 125 low and high grade glioblastoma patient tumor samples for coexpression of CD133 and nestin (Zhang et al., 2008). The authors reported that $CD133^+nestin^+$ was associated with worse outcome and survival, and could potentially be used as independent prognostic indicators. Finally, in 2010, Sato et al. assessed if $CD133^+$ prevalence was associated with spread of the cancer in glioblastoma (Sato et al., 2010). The authors assessed 26 patient samples (16 cases of which the disease had disseminated) and reported that CD133 expression was significantly higher in disseminated disease cases. In summary, these studies agree that initial CD133 expression, especially when assessed in combination with an additional marker, is associated with more aggressive brain cancer and worse outcome. Therefore, the studies provide supportive evidence for the CSC theory postulation that CSCs are the initiators and mediators of cancers. We now await the results of studies evaluating the prognostic potential of the more recently discovered brain CSC markers (i.e. A2B5 and SSEA4) alone and in combination with CD133.

Most recently, the effect of therapy on the CSC population has been evaluated. In 2010, Pallini et al. quantified the frequency of CD133 pre and post radiochemotherapy on 37 paired glioblastoma patient samples (Pallini et al., 2010). In support of the CSC theory that proposes CSCs are resistant to currently employed therapeutics, the researchers noted a significant increase (average 4.6 fold) in $CD133^+$ cells post treatment. However, their analysis further revealed that the increased $CD133^+$ frequency post treatment was surprisingly associated with improved survival, not worse. The authors' following experiments revealed that not all $CD133^+$ cells quantified in the tumors were in actuality tumor cells. The non-tumor cell $CD133^+$ population might potentially have confounded their assessment of CSC frequency pre and post treatment and the effect on patient survival (Pallini et al., 2010). Furthermore, this revelation that not all $CD133^+$ cells are tumor cells may explain their earlier results where $CD133^+$ alone did not predict patient outcome, but $CD133^+Ki67^+$ did (Pallini et al., 2008). These results highlight the importance of employing multiple markers in the accurate identification of a CSC population in illustrating its potential prognostic applicability.

Brain CSC marker	Patient sample size	Prognostic correlation			Publication
		worse outcome	no correlation	improved outcome	
$CD133^+$	95	X			(Zeppernick et al., 2008)
$CD133^+$	36	X			(Beier et al., 2008)
$CD133^+$	44		X		(Pallini et al., 2008)
$CD133^+$	26	X			(Sato et al., 2010)
$CD133^+$	37			X	(Pallini et al., 2010)
$CD133^+Ki67^+$	44	X			(Pallini et al., 2008)
$CD133^+nestin^+$	125	X			(Zhang et al., 2008)

Table 3. Summary of results from immunohistological prognostic studies of brain CSC markers

5. Colon cancer

5.a Identified colon CSC markers

In early 2007, two groups identified a small percentage of highly tumorigenic $CD133^+$ colon cancer (colorectal carcinoma) with the renewal/differentiation properties of CSCs (O'Brien et al., 2007; Ricci-Vitiani et al., 2007). Ricci-Vitiani et al. estimated that CD133+ tumor cells made up 2.5% of total colon tumor cells, and O'Brien further calculated that only 1 in approximately 57,000 colon cancer cells was a CSC, but 1 in 262 CD133+ colon cancer cells was a CSC. Therefore while CD133 was one potential colon CSC marker, there remained others to be identified for the further enrichment of the CSC population. The following year, Shmelkov et al., reported that colon cancer initiating cells (CSCs) were found in both $CD133^+$ and $CD133^-$ tumor cell populations, high-lighting the importance of identifying additional markers (Shmelkov et al., 2008).

Dalerba et al., successfully identified and isolated colon CSCs based on expression of cell surface molecules other than CD133 (Dalerba et al., 2007). The researchers showed that $EpCAM^+CD44^+$ colon cancer cells ranged from 0.03% to 38% (mean = 5.4%) of total colon cancer cells and were highly tumorigenic in immunocompromised mice. In addition, the authors identified another cell surface adhesion molecule, CD166, which could be used for the isolation of colon CSCs. CD166 could be used independently of EpCAM/CD44 or synergistically with these other two markers to further enrich for the CSC population. In 2008, Haraguchi et al. reported that $CD133^+$ colon cancer varied in frequency from 0.3 – 82.5% (mean 35.5%) and that the cancer initiating cell could be further enriched for by isolating cells that were positive for both CD133 and CD44 (Haraguchi et al., 2008). $CD133^+CD44^+$ colon cells were more tumorigenic than $CD133^+$ or $CD44^+$ isolated colon cancer cells. Interestingly, in 2009 another group showed that $CD44^+$ isolated colon cells were highly tumorigenic, but failed to show similar tumorigenicity results when CD133 was used as the selection criterion (Chu et al., 2009).

Spheroid cultured colon CSCs were analysed for their celluar antigen expression profile and were found to be positive for CD133, CD166, CD44, CD29, CD24, Lgr5 and nuclear β-catenin (Vermeulen et al., 2008). All of these were previously known as normal colon stem cell markers, and some had been previously identified as colon CSC markers. The authors further showed that cells identified as $CD133^+CD24^+$ were further enriched for CSCs, but that co-expression of the other identified cell surface markers (CD44, CD166, or CD29) with CD133 failed to further enrich for the CSC population.

With the identification that ALDH activity could be used to isolate breast CSCs (Ginestier et al., 2007), ALDH activity was also assessed as a CSC marker for other solid tumors, including colon cancer. Colon cancer cells isolated based on increased ALDH activity by the aldefluor assay were shown to be more tumorigenic by a number of groups (Carpentino et al., 2009; Chu et al., 2009; Huang et al., 2009). Huang et al. first showed that as few as 25 $ALDH^+$ colon cancer cells could induce tumors in immunocompromised mice, and suggested that ALDH activity may be a more stringent selection marker than CD133 or CD44 for the selection of a colon CSC population (Huang et al., 2009). Undoubtedly, future studies will reveal if $ALDH^+$ combined with expression of these cell surface molecules will lead to further enrichment of the colon CSC population.

5.b Colon CSC markers as prognostic indicators

The data evaluating the use of currently known colon CSC markers as prognostic indicators is mixed and summarized in Table 4. For example, CD133 expression analyses are plentiful

and do not reflect the molecule's prognostic value. We will first review the positive studies. In 2008, Horst et al. performed an immunohistological study of 77 fixed patient tumor samples and found that increased CD133 expression was indicative of worse outcome for patients (Horst et al., 2008). Later in 2009, the same group assessed if expression of CD133 combined with β-catenin had significant prognostic value in a panel of 162 patient samples (Horst et al., 2009a). CD133 and b-catenin stained distinct, partially overlapping cell populations and increased percentages of $CD133^{+}$b-$catenin^{+}$ was a stronger predictor of poor outcome than either marker alone (Horst et al., 2009a). The same group also compared the prognostic value of colon CSC markers CD133, CD44 and CD166 together and alone in a panel of 110 colorectal adenocarcinomas (Horst et al., 2009b). CD133 had the best prognostic potential of the three markers and correlated significantly with worse outcome (Horst et al., 2009b). However, patients with increased $CD133^{+}CD44^{+}CD166^{+}$ tumor cells fared the worse, illustrating again the value of using the markers in combination. In a study by another group, CD133 expression was quantified in 189 colorectal carcinomas and was predictive of worse outcome when specified to patients with well- and moderately-differentiated adenocarcinomas (Kojima et al., 2008). In a final example, increased CD133 expression in a panel of 104 stage IIIB colon carcinoma patient samples correlated with worse prognosis (Li et al., 2009).

In contrast to above described positive results, Choi et al. performed immunohistological assessments on 523 patient samples, that represented the complete range of histoclinical diagnoses, to determine the prognostic value of colon CSC markers CD133, CD44 and CD24 (Choi et al., 2009). Interestingly while expression of CD adhesion molecules correlated with some of the histoclinical prognostic indicators, none were significant prognostic predictors of survival (Choi et al., 2009), disagreeing with the findings of Horst et al. (Horst et al., 2008; Horst et al., 2009a; Horst et al., 2009b). Specifically, the authors determined that CD133 expression correlated with stage, CD24 with degree of differentiation and CD44 with tumor size (Choi et al., 2009). In 2010, Lugli et al., failed to correlate increased CD133 expression with tumor progression or survival time of patients when they probed a large panel of 1420 colorectal cancers by tissue microarray (Lugli et al., 2010). The cohort of samples was also probed for other implicated colon CSC markers; CD166, CD44 and EpCAM, and in contradiction of the CSC theory, their loss of expression, not gain, was associated with increased tumor progression and survival time. This trend was even more evident when the markers were combined (e.g. $CD166^{-}CD44^{-}$).

Independent of the discoveries implicating CD44, CD166, and CD24 as potential colon CSC markers (Dalerba et al., 2007; Vermeulen et al., 2008), the expression of the CD molecules has been previously assessed for predicting the outcome for colorectal cancer patients. For example, expression of certain splice variants of CD44 has been associated with worse outcome for colorectal cancer patients as early as the 1990s. In 1994, Mulder et al., stained 64 patient panel samples and for CD44v6 reported that increased expression of the CD variant was associated with increased tumor-related death (Mulder et al., 1994). However, another study by Weg-Remers et al., failed to detect a correlation between expression of CD44, standard or variants, and patient outcome or tumor progression (Weg-Remers et al., 1998). CD166 expression had been associated with reduced survival, despite not being correlative with tumor grade, stage or nodal involvement (Weichert et al., 2004). The same group later stained a cohort of 147 colon cancer patient samples for CD24 expression and made the distinction between membrane and cytoplasmic CD24 (Weichert et al., 2005). Interestingly, patients with high levels of cyoplasmic CD24 fared significantly worse, being more likely to have higher grade tumors, and develop metastases.

Colon CSC marker	Patient sample size	Prognostic correlation			Publication
		worse outcome	no correlation	improved outcome	
$CD133^+$	77	X			(Horst et al., 2008)
$CD133^+$	189	X			(Kojima et al., 2008)
$CD133^+$	110	X			(Horst et al., 2009b)
$CD133^+$	523		X		(Choi et al., 2009)
$CD133^+$	104	X			(Li et al., 2010a)
$CD133^+$	1420		X		(Lugli et al., 2010)
$CD24^+$	147	X			(Weichert et al., 2005)
$CD24^+$	523		X		(Choi et al., 2009)
$CD44^+$	83		X		(Weg-Remers et al., 1998)
$CD44^+$	110		X		(Horst et al., 2009b)
$CD44^+$	523		X		(Choi et al., 2009)
$CD44^+$	1420			X	(Lugli et al., 2010)
$CD166^+$	111	X			(Weichert et al., 2004)
$CD166^+$	110		X		(Horst et al., 2009b)
$CD166^+$	1420			X	(Lugli et al., 2010)
$EpCam^+$	1420			X	(Lugli et al., 2010)
$CD166^+CD44^+$	1420			X	(Lugli et al., 2010)
$CD133^+$b-catenin$^+$	162	X			(Horst et al., 2009a)
$CD133^+CD44^+CD166^+$	110	X			(Horst et al., 2009b)
$ALDH1A1^+$	1420		X		Lugli, 2010 442 /id}

Table 4. Summary of results from immunohistological prognostic studies of colon CSC markers

Our analysis of the literature reveals a large disparity in the prognostic potential of the identified cell surface colon CSC markers. Potentially, differences in the results between groups could be explained by the varied methods and cut-offs used in tissue staining and scoring (Zlobec et al., 2007). For example Choi et al. scored the stained tissue samples as either positive or negative for expression of the CD molecules, whereas in the studies by Horst et al., the degree of staining was graded as none, low or high (Choi et al., 2009; Horst et al., 2008; Horst et al., 2009a; Horst et al., 2009b). Undoubtedly however, this can only be part of the explanation and it is more likely that the disagreement between groups is potentially an indication of the overall insignificant or poor prognostic value of these CSC markers for colon cancer when employed alone.

With the 2009 discovery that ALDH activity is also specific to colon CSCs (Carpentino et al., 2009; Chu et al., 2009; Huang et al., 2009), the potential of ALDH1A1 as a prognostic indicator is also being evaluated. In the recent study by Lugli et al., described above, who probed a panel of 1420 colorectal carcinomas for currently known cell surface colon CSC markers, the authors also assessed if ALDH1A1 expression had prognostic value (Lugli et al., 2010). The researchers detected ALDH1A1 in less than 25% of samples and failed to correlate patient outcome or disease progression with expression of the protein. Increased ALDH1A1 expression did however correlate with tumor grade (Lugli et al., 2010). In the coming years, the results of more immunohistological studies will clarify the potential prognostic power of ALDH1A1 for colorectal cancer.

6. Prostate cancer

6.a Identified prostate CSC markers

The currently known prostate CSC markers are based on the unique cell surface molecules and functional characteristics of normal prostate stem cells. Combining previously identified prostate stem cell markers $CD44^+$, $\alpha2\beta1^{high}$, $CD133^+$, Collins et al. isolated prostate cancer cells from patient tumor samples that had the *in vitro* self-renewal and differentiation properties of CSCs (Collins et al., 2005). Later in 2006, Patrawala isolated $CD44^+$ prostate cancer cells from cultures and tumors and showed that these cells possessed increased tumorigenicity *in vivo* and had stem cell like qualities (Patrawala et al., 2006). In 2005, using a murine prostate cancer model, Xin et al. showed that prostate cancer cells expressing stem cell antigen-1 (sca-1) were comparatively highly tumorigenic and possessed stem cell like characteristic (Xin et al., 2005). More recently, ALDH activity was also explored as a CSC marker for prostate cancer (Li et al., 2010b; van den Hoogen et al., 2010). Prostate cancer cells with increased ALDH activity were highly tumorigenic and possessed stem cell like characteristics (Li et al., 2010b; van den Hoogen et al., 2010). Interestingly, $ALDH^+$ cancer cells were also positive for CD44 and $\alpha2\beta1$ integrin, but not CD133 (van den Hoogen et al., 2010).

6.b Prostate CSC markers as prognostic indicators

The prognostic potential of currently known prostate CSC markers is ambiguous at best at this time (summarized in Table 5). CD44 has been assessed as a prognostic marker for prostate cancer since the 1990's, long before it was identified as a prostate CSC marker (Patrawala et al., 2006). In 1996, Nagabhushan et al. quantified the prevalence of CD44 in 74 fixed prostate cancer patient samples and noted that CD44 expression correlated inversely with tumor grade (Nagabhushan et al., 1996). A similar inverse relationship was detected in a subsequent study (Noordzij et al., 1997). Then again, 1999 and 2000, the same group

published that CD44 expression decreased in patients with metastatic disease (Noordzij et al., 1999) and the loss of CD44 expression was an independent prognostic predictor of clinical reoccurrence (Vis et al., 2000). In 2001, Aaltomaa et al. analysed 209 prostate cancer samples and found that decreased CD44 expression correlated with metastasis and worse outcome (Aaltomaa et al., 2001). The results of these studies are in clear agreement with the prognostic potential of CD44 for prostate cancer. Unfortunately, from a CSC point of view, they are opposite to the predictions of the CSC theory, whereby an increase in CD44 would be expected to be associated with worse, not better outcomes.

The recent discoveries that ALDH activity could be employed to isolate prostate CSCs were also accompanied by prognostic data. van den Hoogen et al., failed to detect ALDH1A1 in 30 tissue microarray samples and 10 fixed primary tumor samples (van den Hoogen et al., 2010). The authors then decided to evaluate if expression of some of the other ALDH isoforms correlated significantly with clinical pathological determinants. While expression of isoform ALDH7a1 was detected in the majority patient samples, its expression failed to correlate significantly with Gleason score or tumor grade. These findings are in contrast to results published by Li et al. who report that increased ALDH1A1 expression correlated significantly with Gleason score, disease stage, and worse survival (Li et al., 2010b). Future immunohistological studies should resolve the discrepancy between the two groups with regards to the prognostic importance of ALDH1A1.

The greater prognostic potential of employing the CSC markers in combination remains to be shown for prostate cancer. Collins et al. who first discovered that the approximate 0.1%of $CD44^{+}\alpha2\beta1^{high}CD133^{+}$ of all prostate tumor cells had stem cell like characteristics, also reported that prevalence of these potential CSCs did not correlate with tumor grade (Collins et al., 2005). Perhaps future studies combining cell surface and functional markers (e.g. ALDH activity) may reveal a potential prognostic role for prostate CSC markers.

Prostate CSC marker	Patient sample size	Prognostic correlation			Publication
		worse outcome	no correlation	improved outcome	
$CD44^{+}$	74			X	(Nagabhushan et al., 1996)
$CD44^{+}$	97			X	(Noordzij et al., 1997)
$CD44^{+}$	46			X	(Noordzij et al., 1999)
$CD44^{+}$	209			X	(Aaltomaa et al., 2001)
$CD44^{+}\alpha2\beta1^{high}CD133^{+}$	40		X		(Collins et al., 2005)
$ALDH1A1^{+}$	40		X		(van den Hoogen et al., 2010)
$ALDH1A1^{+}$	163	X			(Li et al., 2010b)

Table 5. Summary of results from immunohistological prognostic studies of prostate CSC markers

7. Lung cancer

7.a Identified lung CSC markers

Initially, a side population (SP) of lung cancer cells identified by exclusion of Hoechst 33342 stain were shown to have stem cell like characteristics and overexpression of ABC transporters like ABCG2 was thought to mediate the innate chemo-resistance of stem cells (Hirschmann-Jax et al., 2004). The findings suggested the potential presence of a CSC population in lung cancer. Later, In 2008, Chen et al. isolated $CD133^+$ and $^-$ cancer cell populations from lung cancer cell lines and non-small cell lung cancer patients and reported that $CD133^+$ lung cancer cells had *in vitro* CSC and stem cell like qualities (Chen et al., 2008). This work provided the first indication that CD133 could potentially be used as a lung CSC marker. Later in 2009, Bertolini et al. proved that CD133 was a lung CSC marker (Bertolini et al., 2009). The researchers showed that patient isolated $CD133^+$ (and stained with epithelial-specific antigen to eliminate contaminating cells) lung cancer cells were highly tumorigenic compared to $CD133^-$ cancer cells and had stem cell characteristics (Bertolini et al., 2009). Similar results using CD133 as a lung CSC marker were published by another group later that year, solidifying CD133's recognition as an important lung CSC marker (Tirino et al., 2009).

ALDH activity has also been tested for the isolation of lung CSCs (Jiang et al., 2009; Ucar et al., 2009). Jiang et al. showed it was possible to isolate $ALDH^+$ lung cancer cells from cultured cell lines that were more tumorigenic in immunocompromised mice and displayed stem cell like qualities (i.e. self renewal/differentiation and resistance to chemotherapeutics). As of yet ALDH activity and CD133 have not been employed in combination to potentially isolate a further CSC-encriched population of cells.

7.b Lung CSC markers as prognostic indicators

The data evaluating the use of currently known lung CSC markers as prognostic indicators is mixed and summarized in Table 6. In addition to illustrating the increased tumorigenicity of $CD133^+$ lung cancer cells, Bertolini et al., assessed if CD133 had prognostic value for lung cancer patients (Bertolini et al., 2009). The researchers stained a panel of 42 fixed tumor samples for CD133 expression and showed that patients with $CD133^+$ tumors tended to have a shorter progression-free survival. However, the outcome difference between $CD133^+$ and $CD133^-$ tumors was not statistically significant. Tirino et al. also evaluated the prognostic potential of CD133 for lung cancer (Tirino et al., 2009). Their study of 89 patient samples failed to find a correlation between CD133 expression and the clinical pathological assessments of disease aggressiveness (e.g. tumor size, stage). However they noted a non-significant trend toward shorter disease progression times in the $CD133^+$ patient samples. In a another study of 88 patient samples conducted by Salnikov et al., $CD133^+$ prevalence failed to significantly correlate with tumor size, cancer stage, local metastasis or overall survival (Salnikov et al., 2010). Potentially if a larger sample size was employed in the studies statistical significance may have been reached for some parameters.

The above studies suggest that presence of CD133 alone does not appear to be a strong predictor of disease progression and outcome for lung cancer. However, recent studies employing CD133 in combination with other markers appear more promising. In 2010, Li et al. showed that combination of CD133 with the ABC transporter, ABCG2, was a much more powerful prognostic tool than either marker alone (Li et al., 2010a). The researchers stained a panel of 145 lung cancer patient samples, and when used alone neither marker correlated

significantly with clinical pathological assessment of disease or disease progression. However, when the prevalence of $CD133^+ABCG2^+$ was quantified, increased frequency of $CD133^+ABCG2^+$ cancer cells correlated significantly with shorter times to reoccurrence, illustrating the prognostic power of combining CSC markers.

Finally with their recent discovery that ALDH activity could be employed to isolate lung CSCs, Jiang et al. also determined if ALDH1A1 positivity in lung cancer patient samples was a potential prognostic indicator (Jiang et al., 2009). ALDH1A1 expression correlated significantly with higher tumor grade, disease stage and poor clinical outcome (Jiang et al., 2009). Interestingly, in these immunohistochemical analyses ALDH1A1 positive samples were also $CD133^+$ (60% of patient samples). In contrast, patient samples that were negative for ALDH1A1 expression also lacked CD133 expression. This suggests that potentially $CD133^+$ and $ALDH^+$ can be combined to isolate a more tumorigenic population of lung cancer cells. Future studies will reveal if CD133 combined with ALDH1A1 is a superior and potentially powerful prognostic tool for lung cancer.

Lung CSC marker	Patient sample size	Prognostic correlation			Publication
		worse outcome	no correlation	improved outcome	
$CD133^+$	42		X		(Bertolini et al., 2009)
$CD133^+$	89		X		(Tirino et al., 2009)
$CD133^+$	88		X		(Salnikov et al., 2010)
$CD133^+$	145		X		(Li et al., 2010a)
$ABCG2^+$	145		X		(Li et al., 2010a)
$CD133^+ABCG2^+$	145	X			(Li et al., 2010a)
$ALDH1A1^+$	60	X			(Jiang et al., 2009)

Table 6. Summary of results from immunohistological prognostic studies of lung CSC markers

8. Conclusions

CSCs have become a universal cancer concept. Using *in vitro* and *in vivo* experimental models, this sub-population of highly tumorigenic tumor cells has been shown to exist in most cancers and is resistant to chemo- and radiation therapy. As such, CSCs are believed to be the initiators of cancer, propagators of metastasis and mediators of therapeutic resistance. What is needed is conclusive proof of the importance of CSCs from clinical patient data. As reviewed here, there already exists much clinical data that support or refute the CSC theory from a cancer progression and reoccurrence point of view. Based on publised data thus far, it appears that using a combination of CSC markers, and eliminating the least relevant proposed CSC markers, is the most logical approach not only for accurate identification of CSCs but also for revelation of their important roles in cancer development. With the inevitable future discovery of new CSC markers and their combined use with valid ones previously discovered, the empirical proof that CSCs are the key to both the cause and cure of cancer may be a foregone conclusion.

9. References

Aaltomaa,S., Lipponen,P., la-Opas,M., and Kosma,V.M. (2001). Expression and prognostic value of CD44 standard and variant v3 and v6 isoforms in prostate cancer. Eur. Urol. *39*, 138-144.

Abraham,B.K., Fritz,P., McClellan,M., Hauptvogel,P., Athelogou,M., and Brauch,H. (2005). Prevalence of CD44+/CD24-/low cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. Clin. Cancer Res *11*, 1154-1159.

Advani,A.S., Rodriguez,C., Jin,T., Jawde,R.A., Saber,W., Baz,R., Kalaycio,M., Sobecks,R., Sekeres,M., Tripp,B., and Hsi,E. (2008). Increased C-kit intensity is a poor prognostic factor for progression-free and overall survival in patients with newly diagnosed AML. Leuk. Res. *32*, 913-918.

Al-Hajj,M., Wicha,M.S., ito-Hernandez,A., Morrison,S.J., and Clarke,M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. U. S. A *100*, 3983-3988.

Armstrong,L., Stojkovic,M., Dimmick,I., Ahmad,S., Stojkovic,P., Hole,N., and Lako,M. (2004). Phenotypic characterization of murine primitive hematopoietic progenitor cells isolated on basis of aldehyde dehydrogenase activity. Stem Cells *22*, 1142-1151.

Arslan,O., Akan,H., Beksac,M., Ozcan,M., Koc,H., Ilhan,O., Konuk,N., and Uysal,A. (1996). Lack of prognostic value of CD34 in adult AML. Leuk. Lymphoma *23*, 185-186.

Athanassiadou,P., Grapsa,D., Gonidi,M., Athanassiadou,A.M., Tsipis,A., and Patsouris,E. (2009). CD24 expression has a prognostic impact in breast carcinoma. Pathol. Res. Pract. *205*, 524-533.

Aulmann,S., Waldburger,N., Penzel,R., Andrulis,M., Schirmacher,P., and Sinn,H.P. (2010). Reduction of CD44(+)/CD24(-) breast cancer cells by conventional cytotoxic chemotherapy. Hum. Pathol. *41*, 574-581.

Bankfalvi,A., Terpe,H.J., Breukelmann,D., Bier,B., Rempe,D., Pschadka,G., Krech,R., and Bocker,W. (1998). Gains and losses of CD44 expression during breast carcinogenesis and tumour progression. Histopathology *33*, 107-116.

Barraud,P., Stott,S., Mollgard,K., Parmar,M., and Bjorklund,A. (2007). In vitro characterization of a human neural progenitor cell coexpressing SSEA4 and CD133. J. Neurosci. Res. *85*, 250-259.

Beier,D., Wischhusen,J., Dietmaier,W., Hau,P., Proescholdt,M., Brawanski,A., Bogdahn,U., and Beier,C.P. (2008). CD133 expression and cancer stem cells predict prognosis in high-grade oligodendroglial tumors. Brain Pathol. *18*, 370-377.

Bertolini,G., Roz,L., Perego,P., Tortoreto,M., Fontanella,E., Gatti,L., Pratesi,G., Fabbri,A., Andriani,F., Tinelli,S., Roz,E., Caserini,R., Lo,V.S., Camerini,T., Mariani,L., Delia,D., Calabro,E., Pastorino,U., and Sozzi,G. (2009). Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. Proc. Natl. Acad. Sci. U. S. A *106*, 16281-16286.

Blair,A., Hogge,D.E., Ailles,L.E., Lansdorp,P.M., and Sutherland,H.J. (1997). Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. Blood *89*, 3104-3112.

Blair,A., Hogge,D.E., and Sutherland,H.J. (1998). Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34(+)/CD71(-)/HLA-DR-. Blood *92*, 4325-4335.

Blair,A. and Sutherland,H.J. (2000). Primitive acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo lack surface expression of c-kit (CD117). Exp. Hematol. *28*, 660-671.

Bonnet,D. and Dick,J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. *3*, 730-737.

Borowitz,M.J., Gockerman,J.P., Moore,J.O., Civin,C.I., Page,S.O., Robertson,J., and Bigner,S.H. (1989). Clinicopathologic and cytogenic features of CD34 (My 10)-positive acute nonlymphocytic leukemia. Am. J. Clin. Pathol. *91*, 265-270.

Bradstock,K., Matthews,J., Benson,E., Page,F., and Bishop,J. (1994). Prognostic value of immunophenotyping in acute myeloid leukemia. Australian Leukaemia Study Group. Blood *84*, 1220-1225.

Campos,L., Guyotat,D., Archimbaud,E., Calmard-Oriol,P., Tsuruo,T., Troncy,J., Treille,D., and Fiere,D. (1992). Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. Blood *79*, 473-476.

Campos,L., Guyotat,D., Archimbaud,E., Devaux,Y., Treille,D., Larese,A., Maupas,J., Gentilhomme,O., Ehrsam,A., and Fiere,D. (1989). Surface marker expression in adult acute myeloid leukaemia: correlations with initial characteristics, morphology and response to therapy. Br. J. Haematol. *72*, 161-166.

Carpentino,J.E., Hynes,M.J., Appelman,H.D., Zheng,T., Steindler,D.A., Scott,E.W., and Huang,E.H. (2009). Aldehyde dehydrogenase-expressing colon stem cells contribute to tumorigenesis in the transition from colitis to cancer. Cancer Res. *69*, 8208-8215.

Charafe-Jauffret,E., Ginestier,C., Iovino,F., Tarpin,C., Diebel,M., Esterni,B., Houvenaeghel,G., Extra,J.M., Bertucci,F., Jacquemier,J., Xerri,L., Dontu,G., Stassi,G., Xiao,Y., Barsky,S.H., Birnbaum,D., Viens,P., and Wicha,M.S. (2010). Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clin. Cancer Res. *16*, 45-55.

Chen,Y.C., Hsu,H.S., Chen,Y.W., Tsai,T.H., How,C.K., Wang,C.Y., Hung,S.C., Chang,Y.L., Tsai,M.L., Lee,Y.Y., Ku,H.H., and Chiou,S.H. (2008). Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PLoS. ONE. *3*, e2637.

Cheung,A.M., Wan,T.S., Leung,J.C., Chan,L.Y., Huang,H., Kwong,Y.L., Liang,R., and Leung,A.Y. (2007). Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. Leukemia *21*, 1423-1430.

Choi,D., Lee,H.W., Hur,K.Y., Kim,J.J., Park,G.S., Jang,S.H., Song,Y.S., Jang,K.S., and Paik,S.S. (2009). Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. World J. Gastroenterol. *15*, 2258-2264.

Chu,P., Clanton,D.J., Snipas,T.S., Lee,J., Mitchell,E., Nguyen,M.L., Hare,E., and Peach,R.J. (2009). Characterization of a subpopulation of colon cancer cells with stem cell-like properties. Int. J. Cancer *124*, 1312-1321.

Chute,J.P., Muramoto,G.G., Whitesides,J., Colvin,M., Safi,R., Chao,N.J., and McDonnell,D.P. (2006). Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. Proc. Natl. Acad. Sci. U. S. A *103*, 11707-11712.

Ciolli,S., Leoni,F., Caporale,R., Pascarella,A., Salti,F., and Rossi-Ferrini,P. (1993). CD34 expression fails to predict the outcome in adult acute myeloid leukemia. Haematologica *78*, 151-155.

Collins,A.T., Berry,P.A., Hyde,C., Stower,M.J., and Maitland,N.J. (2005). Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res *65*, 10946-10951.

Collins,S.J. (2008). Retinoic acid receptors, hematopoiesis and leukemogenesis. Curr. Opin. Hematol. *15*, 346-351.

Dalal,B.I., Wu,V., Barnett,M.J., Horsman,D.E., Spinelli,J.J., Naiman,S.C., Shepherd,J.D., Nantel,S.H., Reece,D.E., Sutherland,H.J., Klingemann,H.G., and Phillips,G.L. (1997). Induction failure in de novo acute myelogenous leukemia is associated with expression of high levels of CD34 antigen by the leukemic blasts. Leuk. Lymphoma *26*, 299-306.

Dalerba,P., Dylla,S.J., Park,I.K., Liu,R., Wang,X., Cho,R.W., Hoey,T., Gurney,A., Huang,E.H., Simeone,D.M., Shelton,A.A., Parmiani,G., Castelli,C., and Clarke,M.F. (2007). Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. U. S. A *104*, 10158-10163.

Dalton,L.W., Pinder,S.E., Elston,C.E., Ellis,I.O., Page,D.L., Dupont,W.D., and Blamey,R.W. (2000). Histologic grading of breast cancer: linkage of patient outcome with level of pathologist agreement. Mod. Pathol. *13*, 730-735.

Ebinger,M., Witte,K.E., Ahlers,J., Schafer,I., Andre,M., Kerst,G., Scheel-Walter,H.G., Lang,P., and Handgretinger,R. (2010). High frequency of immature cells at diagnosis predicts high minimal residual disease level in childhood acute lymphoblastic leukemia. Leuk. Res. *34*, 1139-1142.

Fagioli,F., Cuneo,A., Carli,M.G., Bardi,A., Piva,N., Previati,R., Rigolin,G.M., Ferrari,L., Spanedda,R., and Castoldi,G. (1993). Chromosome aberrations in CD34-positive acute myeloid leukemia. Correlation with clinicopathologic features. Cancer Genet. Cytogenet. *71*, 119-124.

Fruchart,C., Lenormand,B., Bastard,C., Boulet,D., Lesesve,J.F., Callat,M.P., Stamatoullas,A., Monconduit,M., and Tilly,H. (1996). Correlation between CD34 expression and chromosomal abnormalities but not clinical outcome in acute myeloid leukemia. Am. J. Hematol. *53*, 175-180.

Geller,R.B., Zahurak,M., Hurwitz,C.A., Burke,P.J., Karp,J.E., Piantadosi,S., and Civin,C.I. (1990). Prognostic importance of immunophenotyping in adults with acute myelocytic leukaemia: the significance of the stem-cell glycoprotein CD34 (My10). Br. J. Haematol. *76*, 340-347.

Ginestier,C., Hur,M.H., Charafe-Jauffret,E., Monville,F., Dutcher,J., Brown,M., Jacquemier,J., Viens,P., Kleer,C.G., Liu,S., Schott,A., Hayes,D., Birnbaum,D., Wicha,M.S., and Dontu,G. (2007). ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome. Cell Stem Cell *1*, 555-567.

Guinot,M., Sanz,G.F., Sempere,A., Arilla,M.J., Jarque,I., Gomis,F., and Sanz,M.A. (1991). Prognostic value of CD34 expression in de novo acute myeloblastic leukaemia. Br. J. Haematol. *79*, 533-534.

Haraguchi,N., Ohkuma,M., Sakashita,H., Matsuzaki,S., Tanaka,F., Mimori,K., Kamohara,Y., Inoue,H., and Mori,M. (2008). CD133+CD44+ population efficiently enriches colon cancer initiating cells. Ann. Surg. Oncol. *15*, 2927-2933.

Hirschmann-Jax,C., Foster,A.E., Wulf,G.G., Nuchtern,J.G., Jax,T.W., Gobel,U., Goodell,M.A., and Brenner,M.K. (2004). A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc. Natl. Acad. Sci. U. S. A *101*, 14228-14233.

Honeth,G., Bendahl,P.O., Ringner,M., Saal,L.H., Gruvberger-Saal,S.K., Lovgren,K., Grabau,D., Ferno,M., Borg,A., and Hegardt,C. (2008). The CD44+/CD24- phenotype is enriched in basal-like breast tumors. Breast Cancer Res. *10*, R53.

Horst,D., Kriegl,L., Engel,J., Jung,A., and Kirchner,T. (2009a). CD133 and nuclear beta-catenin: the marker combination to detect high risk cases of low stage colorectal cancer. Eur. J. Cancer *45*, 2034-2040.

Horst,D., Kriegl,L., Engel,J., Kirchner,T., and Jung,A. (2008). CD133 expression is an independent prognostic marker for low survival in colorectal cancer. Br. J. Cancer *99*, 1285-1289.

Horst,D., Kriegl,L., Engel,J., Kirchner,T., and Jung,A. (2009b). Prognostic significance of the cancer stem cell markers CD133, CD44, and CD166 in colorectal cancer. Cancer Invest *27*, 844-850.

Huang,E.H., Hynes,M.J., Zhang,T., Ginestier,C., Dontu,G., Appelman,H., Fields,J.Z., Wicha,M.S., and Boman,B.M. (2009). Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. Cancer Res. *69*, 3382-3389.

Jiang,F., Qiu,Q., Khanna,A., Todd,N.W., Deepak,J., Xing,L., Wang,H., Liu,Z., Su,Y., Stass,S.A., and Katz,R.L. (2009). Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. Mol. Cancer Res. *7*, 330-338.

Joensuu,H., Klemi,P.J., Toikkanen,S., and Jalkanen,S. (1993). Glycoprotein CD44 expression and its association with survival in breast cancer. Am. J. Pathol. *143*, 867-874.

Jordan,C.T., Upchurch,D., Szilvassy,S.J., Guzman,M.L., Howard,D.S., Pettigrew,A.L., Meyerrose,T., Rossi,R., Grimes,B., Rizzieri,D.A., Luger,S.M., and Phillips,G.L. (2000). The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia *14*, 1777-1784.

Kanda,Y., Hamaki,T., Yamamoto,R., Chizuka,A., Suguro,M., Matsuyama,T., Takezako,N., Miwa,A., Kami,M., Hirai,H., and Togawa,A. (2000). The clinical significance of CD34 expression in response to therapy of patients with acute myeloid leukemia: an overview of 2483 patients from 22 studies. Cancer *88*, 2529-2533.

Keyhani,A., Huh,Y.O., Jendiroba,D., Pagliaro,L., Cortez,J., Pierce,S., Pearlman,M., Estey,E., Kantarjian,H., and Freireich,E.J. (2000). Increased CD38 expression is associated with favorable prognosis in adult acute leukemia. Leuk. Res. *24*, 153-159.

Koehler,M., Behm,F., Hancock,M., and Pui,C.H. (1993). Expression of activation antigens CD38 and CD71 is not clinically important in childhood acute lymphoblastic leukemia. Leukemia *7*, 41-45.

Kojima,M., Ishii,G., Atsumi,N., Fujii,S., Saito,N., and Ochiai,A. (2008). Immunohistochemical detection of CD133 expression in colorectal cancer: a clinicopathological study. Cancer Sci. *99*, 1578-1583.

Kristiansen,G., Winzer,K.J., Mayordomo,E., Bellach,J., Schluns,K., Denkert,C., Dahl,E., Pilarsky,C., Altevogt,P., Guski,H., and Dietel,M. (2003). CD24 expression is a new prognostic marker in breast cancer. Clin. Cancer Res. *9*, 4906-4913.

Kyoda,K., Nakamura,S., Hattori,N., Takeshima,M., Nakamura,K., Kaya,H., Matano,S., Okumura,H., Kanno,M., Ohtake,S., and Matsuda,T. (1998). Lack of prognostic

significance of CD34 expression in adult AML when FAB M0 and M3 are excluded. Am. J. Hematol. *57*, 265-266.

Lamy,T., Goasguen,J.E., Mordelet,E., Grulois,I., Dauriac,C., Drenou,B., Chaperon,J., Fauchet,R., and le Prise,P.Y. (1994). P-glycoprotein (P-170) and CD34 expression in adult acute myeloid leukemia (AML). Leukemia *8*, 1879-1883.

Lanzkron,S.M., Collector,M.I., and Sharkis,S.J. (1999). Hematopoietic stem cell tracking in vivo: a comparison of short-term and long-term repopulating cells. Blood *93*, 1916-1921.

Lapidot,T., Sirard,C., Vormoor,J., Murdoch,B., Hoang,T., Caceres-Cortes,J., Minden,M., Paterson,B., Caligiuri,M.A., and Dick,J.E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature *367*, 645-648.

Lee,E.J., Yang,J., Leavitt,R.D., Testa,J.R., Civin,C.I., Forrest,A., and Schiffer,C.A. (1992). The significance of CD34 and TdT determinations in patients with untreated de novo acute myeloid leukemia. Leukemia *6*, 1203-1209.

Li,C.Y., Li,B.X., Liang,Y., Peng,R.Q., Ding,Y., Xu,D.Z., Zhang,X., Pan,Z.Z., Wan,D.S., Zeng,Y.X., Zhu,X.F., and Zhang,X.S. (2009). Higher percentage of CD133+ cells is associated with poor prognosis in colon carcinoma patients with stage IIIB. J. Transl. Med. *7*, 56.

Li,F., Zeng,H., and Ying,K. (2010a). The combination of stem cell markers CD133 and ABCG2 predicts relapse in stage I non-small cell lung carcinomas. Med. Oncol.

Li,T., Su,Y., Mei,Y., Leng,Q., Leng,B., Liu,Z., Stass,S.A., and Jiang,F. (2010b). ALDH1A1 is a marker for malignant prostate stem cells and predictor of prostate cancer patients' outcome. Lab Invest *90*, 234-244.

Looi,L.M., Cheah,P.L., Zhao,W., Ng,M.H., and Yip,C.H. (2006). CD44 expression and axillary lymph node metastasis in infiltrating ductal carcinoma of the breast. Malays. J. Pathol. *28*, 83-86.

Lugli,A., Iezzi,G., Hostettler,I., Muraro,M.G., Mele,V., Tornillo,L., Carafa,V., Spagnoli,G., Terracciano,L., and Zlobec,I. (2010). Prognostic impact of the expression of putative cancer stem cell markers CD133, CD166, CD44s, EpCAM, and ALDH1 in colorectal cancer. Br. J. Cancer *103*, 382-390.

Magni,M., Shammah,S., Schiro,R., Mellado,W., la-Favera,R., and Gianni,A.M. (1996). Induction of cyclophosphamide-resistance by aldehyde-dehydrogenase gene transfer. Blood *87*, 1097-1103.

Marchitti,S.A., Brocker,C., Stagos,D., and Vasiliou,V. (2008). Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. Expert. Opin. Drug Metab Toxicol. *4*, 697-720.

McSherry,E.A., Donatello,S., Hopkins,A.M., and McDonnell,S. (2007). Molecular basis of invasion in breast cancer. Cell Mol. Life Sci. 64, 3201-18.

Morimoto,K., Kim,S.J., Tanei,T., Shimazu,K., Tanji,Y., Taguchi,T., Tamaki,Y., Terada,N., and Noguchi,S. (2009). Stem cell marker aldehyde dehydrogenase 1-positive breast cancers are characterized by negative estrogen receptor, positive human epidermal growth factor receptor type 2, and high Ki67 expression. Cancer Sci. *100*, 1062-1068.

Mulder,J.W., Kruyt,P.M., Sewnath,M., Oosting,J., Seldenrijk,C.A., Weidema,W.F., Offerhaus,G.J., and Pals,S.T. (1994). Colorectal cancer prognosis and expression of exon-v6-containing CD44 proteins. Lancet *344*, 1470-1472.

Myint,H. and Lucie,N.P. (1992). The prognostic significance of the CD34 antigen in acute myeloid leukaemia. Leuk. Lymphoma *7*, 425-429.

Mylona,E., Giannopoulou,I., Fasomytakis,E., Nomikos,A., Magkou,C., Bakarakos,P., and Nakopoulou,L. (2008). The clinicopathologic and prognostic significance of CD44+/CD24(-/low) and CD44-/CD24+ tumor cells in invasive breast carcinomas. Hum. Pathol. *39*, 1096-1102.

Nagabhushan,M., Pretlow,T.G., Guo,Y.J., Amini,S.B., Pretlow,T.P., and Sy,M.S. (1996). Altered expression of CD44 in human prostate cancer during progression. Am. J. Clin. Pathol. *106*, 647-651.

Neumeister,V., Agarwal,S., Bordeaux,J., Camp,R.L., and Rimm,D.L. (2010). In situ identification of putative cancer stem cells by multiplexing ALDH1, CD44, and cytokeratin identifies breast cancer patients with poor prognosis. Am. J. Pathol. *176*, 2131-2138.

Neumeister,V. and Rimm,D. (2010). Is ALDH1 a good method for definition of breast cancer stem cells? Breast Cancer Res. Treat. 123, 109-11.

Noordzij,M.A., van Steenbrugge,G.J., Schroder,F.H., and van der Kwast,T.H. (1999). Decreased expression of CD44 in metastatic prostate cancer. Int. J. Cancer *84*, 478-483.

Noordzij,M.A., van Steenbrugge,G.J., Verkaik,N.S., Schroder,F.H., and van der Kwast,T.H. (1997). The prognostic value of CD44 isoforms in prostate cancer patients treated by radical prostatectomy. Clin. Cancer Res. *3*, 805-815.

Nunes,M.C., Roy,N.S., Keyoung,H.M., Goodman,R.R., McKhann,G., Jiang,L., Kang,J., Nedergaard,M., and Goldman,S.A. (2003). Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nat. Med. *9*, 439-447.

O'Brien,C.A., Pollett,A., Gallinger,S., and Dick,J.E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature *445*, 106-110.

Ogden,A.T., Waziri,A.E., Lochhead,R.A., Fusco,D., Lopez,K., Ellis,J.A., Kang,J., Assanah,M., McKhann,G.M., Sisti,M.B., McCormick,P.C., Canoll,P., and Bruce,J.N. (2008). Identification of A2B5+CD133- tumor-initiating cells in adult human gliomas. Neurosurgery *62*, 505-514.

Ozer,E., Canda,T., and Kurtodlu,B. (1997). The role of angiogenesis, laminin and CD44 expression in metastatic behavior of early-stage low-grade invasive breast carcinomas. Cancer Lett. *121*, 119-123.

Pallini,R., Ricci-Vitiani,L., Banna,G.L., Signore,M., Lombardi,D., Todaro,M., Stassi,G., Martini,M., Maira,G., Larocca,L.M., and De,M.R. (2008). Cancer stem cell analysis and clinical outcome in patients with glioblastoma multiforme. Clin. Cancer Res. *14*, 8205-8212.

Pallini,R., Ricci-Vitiani,L., Montano,N., Mollinari,C., Biffoni,M., Cenci,T., Pierconti,F., Martini,M., De,M.R., and Larocca,L.M. (2010). Expression of the stem cell marker CD133 in recurrent glioblastoma and its value for prognosis. Cancer.

Patrawala,L., Calhoun,T., Schneider-Broussard,R., Li,H., Bhatia,B., Tang,S., Reilly,J.G., Chandra,D., Zhou,J., Claypool,K., Coghlan,L., and Tang,D.G. (2006). Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene *25*, 1696-1708.

Pece,S., Tosoni,D., Confalonieri,S., Mazzarol,G., Vecchi,M., Ronzoni,S., Bernard,L., Viale,G., Pelicci,P.G., and Di Fiore,P.P. (2010). Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell *140*, 62-73.

Phillips,T.M., McBride,W.H., and Pajonk,F. (2006). The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J. Natl. Cancer Inst. *98*, 1777-1785.

Porwit-MacDonald,A., Janossy,G., Ivory,K., Swirsky,D., Peters,R., Wheatley,K., Walker,H., Turker,A., Goldstone,A.H., and Burnett,A. (1996). Leukemia-associated changes identified by quantitative flow cytometry. IV. CD34 overexpression in acute myelogenous leukemia M2 with t(8;21). Blood *87*, 1162-1169.

Ran,D., Schubert,M., Pietsch,L., Taubert,I., Wuchter,P., Eckstein,V., Bruckner,T., Zoeller,M., and Ho,A.D. (2009). Aldehyde dehydrogenase activity among primary leukemia cells is associated with stem cell features and correlates with adverse clinical outcomes. Exp. Hematol. *37*, 1423-1434.

Raspadori,D., Lauria,F., Ventura,M.A., Rondelli,D., Visani,G., de,V.A., and Tura,S. (1997). Incidence and prognostic relevance of CD34 expression in acute myeloblastic leukemia: analysis of 141 cases. Leuk. Res. *21*, 603-607.

Repp,R., Schaekel,U., Helm,G., Thiede,C., Soucek,S., Pascheberg,U., Wandt,H., Aulitzky,W., Bodenstein,H., Sonnen,R., Link,H., Ehninger,G., and Gramatzki,M. (2003). Immunophenotyping is an independent factor for risk stratification in AML. Cytometry B Clin. Cytom. *53*, 11-19.

Resetkova,E., Reis-Filho,J.S., Jain,R.K., Mehta,R., Thorat,M.A., Nakshatri,H., and Badve,S. (2010). Prognostic impact of ALDH1 in breast cancer: a story of stem cells and tumor microenvironment. Breast Cancer Res. Treat. 123, 97-108.

Ricci-Vitiani,L., Lombardi,D.G., Pilozzi,E., Biffoni,M., Todaro,M., Peschle,C., and De,M.R. (2007). Identification and expansion of human colon-cancer-initiating cells. Nature *445*, 111-115.

Salnikov,A.V., Gladkich,J., Moldenhauer,G., Volm,M., Mattern,J., and Herr,I. (2010). CD133 is indicative for a resistance phenotype but does not represent a prognostic marker for survival of non-small cell lung cancer patients. Int. J. Cancer *126*, 950-958.

Sato,A., Sakurada,K., Kumabe,T., Sasajima,T., Beppu,T., Asano,K., Ohkuma,H., Ogawa,A., Mizoi,K., Tominaga,T., Kitanaka,C., and Kayama,T. (2010). Association of stem cell marker CD133 expression with dissemination of glioblastomas. Neurosurg. Rev. *33*, 175-183.

Schneider,J., Pollan,M., Ruibal,A., Jimenez,E., Lucas,A.R., Nunez,M.I., Sanchez,J., and Tejerina,A. (1999). Histologic grade and CD44 are independent predictors of axillary lymph node invasion in early (T1) breast cancer. Tumour. Biol. *20*, 319-330.

Selleri,C., Notaro,R., Catalano,L., Fontana,R., Del,V.L., and Rotoli,B. (1992). Prognostic irrelevance of CD34 in acute myeloid leukaemia. Br. J. Haematol. *82*, 479-481.

Shmelkov,S.V., Butler,J.M., Hooper,A.T., Hormigo,A., Kushner,J., Milde,T., St,C.R., Baljevic,M., White,I., Jin,D.K., Chadburn,A., Murphy,A.J., Valenzuela,D.M., Gale,N.W., Thurston,G., Yancopoulos,G.D., D'Angelica,M., Kemeny,N., Lyden,D., and Rafii,S. (2008). CD133 expression is not restricted to stem cells, and both CD133+ and C. J. Clin. Invest *118*, 2111-2120.

Singh,S.K., Clarke,I.D., Terasaki,M., Bonn,V.E., Hawkins,C., Squire,J., and Dirks,P.B. (2003). Identification of a cancer stem cell in human brain tumors. Cancer Res. *63*, 5821-5828.

Singh,S.K., Hawkins,C., Clarke,I.D., Squire,J.A., Bayani,J., Hide,T., Henkelman,R.M., Cusimano,M.D., and Dirks,P.B. (2004). Identification of human brain tumour initiating cells. Nature *432*, 396-401.

Solary,E., Casasnovas,R.O., Campos,L., Bene,M.C., Faure,G., Maingon,P., Falkenrodt,A., Lenormand,B., and Genetet,N. (1992). Surface markers in adult acute myeloblastic leukemia: correlation of CD19+, CD34+ and CD14+/DR--phenotypes with shorter survival. Groupe d'Etude Immunologique des Leucemies (GEIL). Leukemia *6*, 393-399.

Son,M.J., Woolard,K., Nam,D.H., Lee,J., and Fine,H.A. (2009). SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell *4*, 440-452.

Sperling,C., Buchner,T., Creutzig,U., Ritter,J., Harbott,J., Fonatsch,C., Sauerland,C., Mielcarek,M., Maschmeyer,G., Loffler,H., and . (1995). Clinical, morphologic, cytogenetic and prognostic implications of CD34 expression in childhood and adult de novo AML. Leuk. Lymphoma *17*, 417-426.

Tchoghandjian,A., Baeza,N., Colin,C., Cayre,M., Metellus,P., Beclin,C., Ouafik,L., and Figarella-Branger,D. (2010). A2B5 cells from human glioblastoma have cancer stem cell properties. Brain Pathol. *20*, 211-221.

te Boekhorst,P.A., de,L.K., Schoester,M., Wittebol,S., Nooter,K., Hagemeijer,A., Lowenberg,B., and Sonneveld,P. (1993). Predominance of functional multidrug resistance (MDR-1) phenotype in CD34+ acute myeloid leukemia cells. Blood *82*, 3157-3162.

Tirino,V., Camerlingo,R., Franco,R., Malanga,D., La,R.A., Viglietto,G., Rocco,G., and Pirozzi,G. (2009). The role of CD133 in the identification and characterisation of tumour-initiating cells in non-small-cell lung cancer. Eur. J. Cardiothorac. Surg. *36*, 446-453.

Ucar,D., Cogle,C.R., Zucali,J.R., Ostmark,B., Scott,E.W., Zori,R., Gray,B.A., and Moreb,J.S. (2009). Aldehyde dehydrogenase activity as a functional marker for lung cancer. Chem. Biol. Interact. *178*, 48-55.

Uchida,N., Buck,D.W., He,D., Reitsma,M.J., Masek,M., Phan,T.V., Tsukamoto,A.S., Gage,F.H., and Weissman,I.L. (2000). Direct isolation of human central nervous system stem cells. Proc. Natl. Acad. Sci. U. S. A *97*, 14720-14725.

van den Hoogen,C., van der,H.G., Cheung,H., Buijs,J.T., Lippitt,J.M., Guzman-Ramirez,N., Hamdy,F.C., Eaton,C.L., Thalmann,G.N., Cecchini,M.G., Pelger,R.C., and van der,P.G. (2010). High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. Cancer Res. *70*, 5163-5173.

van Rhenen A., Feller,N., Kelder,A., Westra,A.H., Rombouts,E., Zweegman,S., van der Pol,M.A., Waisfisz,Q., Ossenkoppele,G.J., and Schuurhuis,G.J. (2005). High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin. Cancer Res. *11*, 6520-6527.

Vermeulen,L., Todaro,M., de Sousa,M.F., Sprick,M.R., Kemper,K., Perez,A.M., Richel,D.J., Stassi,G., and Medema,J.P. (2008). Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. Proc. Natl. Acad. Sci. U. S. A *105*, 13427-13432.

Vis,A.N., Noordzij,M.A., Fitoz,K., Wildhagen,M.F., Schroder,F.H., and van der Kwast,T.H. (2000). Prognostic value of cell cycle proteins p27(kip1) and MIB-1, and the cell

adhesion protein CD44s in surgically treated patients with prostate cancer. J. Urol. *164*, 2156-2161.

Weg-Remers,S., Anders,M., von,L.B., Riecken,E.O., Schuder,G., Feifel,G., Zeitz,M., and Stallmach,A. (1998). Decreased expression of CD44 splicing variants in advanced colorectal carcinomas. Eur. J. Cancer *34*, 1607-1611.

Weichert,W., Denkert,C., Burkhardt,M., Gansukh,T., Bellach,J., Altevogt,P., Dietel,M., and Kristiansen,G. (2005). Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. Clin. Cancer Res. *11*, 6574-6581.

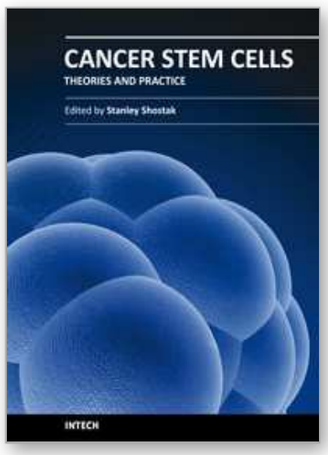
Weichert,W., Knosel,T., Bellach,J., Dietel,M., and Kristiansen,G. (2004). ALCAM/CD166 is overexpressed in colorectal carcinoma and correlates with shortened patient survival. J. Clin. Pathol. *57*, 1160-1164.

Xin,L., Lawson,D.A., and Witte,O.N. (2005). The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. Proc. Natl. Acad. Sci. U. S. A *102*, 6942-6947.

Zeppernick,F., Ahmadi,R., Campos,B., Dictus,C., Helmke,B.M., Becker,N., Lichter,P., Unterberg,A., Radlwimmer,B., and Herold-Mende,C.C. (2008). Stem cell marker CD133 affects clinical outcome in glioma patients. Clin. Cancer Res. *14*, 123-129.

Zhang,M., Song,T., Yang,L., Chen,R., Wu,L., Yang,Z., and Fang,J. (2008). Nestin and CD133: valuable stem cell-specific markers for determining clinical outcome of glioma patients. J. Exp. Clin. Cancer Res. *27*, 85.

Zlobec,I., Terracciano,L., Jass,J.R., and Lugli,A. (2007). Value of staining intensity in the interpretation of immunohistochemistry for tumor markers in colorectal cancer. Virchows Arch. *451*, 763-769



Cancer Stem Cells Theories and Practice

Edited by Prof. Stanley Shostak

ISBN 978-953-307-225-8
Hard cover, 442 pages
Publisher InTech
Published online 22, March, 2011
Published in print edition March, 2011

Cancer Stem Cells Theories and Practice does not 'boldly go where no one has gone before!' Rather, Cancer Stem Cells Theories and Practice boldly goes where the cutting edge of research theory meets the concrete challenges of clinical practice. Cancer Stem Cells Theories and Practice is firmly grounded in the latest results on cancer stem cells (CSCs) from world-class cancer research laboratories, but its twenty-two chapters also tease apart cancer's vulnerabilities and identify opportunities for early detection, targeted therapy, and reducing remission and resistance.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen