RATIONALE OF THE STUDY:

Despite the recent advances in diagnosis and therapeutic modalities, prognostic rate remains dismal with over half of the oral cancer patients suffering from loco-regional relapses while 1/4th of the patients developing distant organ metastases [277, 191]. Thus, it is becoming increasingly important to develop methods for monitoring disease progression and detecting early metastatic spread in order to facilitate individual therapeutic interventions. Circulating tumor cells (CTCs), a subpopulation of rare atypical precursor cells released in the circulation by primary tumors, are believed to have acquired somatic mutations and genomic rearrangements identical to that found in primary tumors. This characteristic of CTCs would aid in understanding the mutational behavioural pattern of tumors without the need for an invasive tissue biopsy [278]. Recent evidences have demonstrated significant correlations between occurrence of CTCs and disease progression, poor prognosis and disease free survival in breast, prostate, colorectal, and lung cancers; however the prognostic value and clinical utility of CTCs in OSCC is yet to be elucidated [279, 280]. Inspite of the impending potential of CTCs, there are certain challenges that need to be considered such as - (i) Reusability of the processed samples (ii) Short life span of CTCs in circulation and (iii) Plausible spreading of disseminating cells during surgery or incision biopsy [280, 281]. Thus, overcoming these obstacles would not only facilitate monitoring the therapeutic response in patients but would also help identify potential targets for individual patient therapy.

Recent reports have observed a great discrepancy in correlation of CTC status with clinico-pathological characteristics of cancer patients due to usage of different techniques such as - Immunomagnetic separation, Microfluidic platforms and cellular-marker based enrichment [161, 190, 282, 283]. Amongst these, EpCAM based enrichment technique has been one of the most widely used techniques till date; however it could not detect CTCs in all metastasized or advanced disease patients of OSCC. This unpredictability is consistent with other reports and could be attributed to either low incidence of CTCs or to exclusion of non-EpCAM and/or non-keratin expressing CTCs which needs serious contemplation as EMT is characterised by loss of epithelial features [284]. Hence, there was a paradigm shift from EpCAM based enrichment techniques to technologies capable of capturing non-EpCAM based CTC population by using other cell surface markers (N-cadherin and EGFR) or cancer stem
cell (CSC) markers (CD44) [285, 286]. Mani et al and Yu et al observed that amongst the probable set of markers, stem like cells demonstrated significant dynamic changes and their ability to survive in circulation was considerably higher owing to its self-renewal property [139, 287]. Furthermore, culturing CTCs based on CSCs markers has become an imperative aspect to develop in-vitro models for assessing drug sensitivity and improvising personalized treatment; however this approach is less explored in OSCC [280]. Therefore, this study aims to understand the molecular diversity and critical role of this disseminated tumor population in OSCC by culturing patient derived CD44+ circulatory tumor stem like cells ex-vivo. Furthermore, we sought to assess the cytotoxic effect of Cisplatin on this rare subpopulation, evaluate the gene expression profile of pertinent CSC markers and analyze their clinical significance in OSCC patients, thus providing a better model to monitor disease progression, therapeutic response and identify potential targets for personalised therapy in future.
METHODOLOGY:

**OSCC tissues collection and processing:**

Peripheral Blood Samples of 30 OSCC Patients (23 tobacco habituated and 7 non habituated) and 15 healthy controls were collected for the study using EDTA vacuum tubes (Becton Dickinson Vacutainer Systems Europe, Meylan, France). Whole blood samples were processed fresh on the same day for RNA isolation using RNA blood mini kit (Qiagen) following manufacturer’s instructions. This study was approved by the Ethical Committee of The Gujarat Cancer & Research Institute, Gujarat and written informed consent were duly taken from the patients for participation in the study.

**Immuno-magnetic cell sorting of cancer stem cells and cell culture:**

Mononuclear cells were isolated from peripheral blood of OSCC patients by density gradient centrifugation. These cells were exposed to FITC-conjugated anti-CD44 mouse antibody (Stem Cell Technologies, USA) to identify the CD44+ cells from the heterogeneous tumor population while the remaining cells apart from the positive subpopulation was considered as the CD44- subpopulation. Further, EasySep® FITC positive selection kit (Stem Cell Technologies, USA) was used to identify the FITC labelled CD44 positive cells. These sorted cells were cultured in RPMI (HiMedia), 10% fetal bovine serum (FBS; HiMedia), and 100 U/ml Penicillin-streptomycin (HiMedia) in low attachment conditions. All experiments were performed in duplicates to verify the reproducibility of the data.

**Flow cytometry:**

The purity of immuno-magnetically sorted CD44+ subpopulation was validated by gating the sorted cells with conjugated primary antibodies of CD44-FITC (Stem Cell Technologies, USA), CD24-PE and CD45-PE (BD Biosystems) phenotypes using flow cytometry analysis. These cells were exposed to anti-mouse secondary antibodies and percentage of each population was calculated by acquiring the sample in FACs Canto II instrument using FACS Diva software.
**Sphere Forming Assay:**

The immuno-magnetically-sorted cells were seeded in 6-well ultra-low attachment plates (Corning; New York, NY, USA) at a density of $5 \times 10^3$ cells/well and cultured in RPMI-1640, 10% FBS and 100 U/ml Penicillin-streptomycin at 37°C and 5% CO$_2$. The percentage of orosphere was calculated by dividing the number of spheres by the number of cells seeded per well.

**Drug cytotoxicity assay:**

CD44+ and CD44- subpopulations were seeded at a density of $1 \times 10^4$ in 96-well plate. These cells were exposed to cisplatin drug for 24 hrs in various concentrations ranging from 0.25 - 1.0 μg/ml. Further, 10μl of 5 mg/ml MTT (Hi-Media) was added to each well and the cells were incubated at 37°C for 4 h. 200μl of DMSO was added and mixed thoroughly on orbital shaker for 15 minutes to dissolve the formazan crystals. Absorbance was estimated at 590 nm with a reference filter of 620 nm using an ELISA reader (Multiskan Spectrum Microplate Reader, Thermo Scientific). The experiments were performed in triplicates. All data were normalized to corresponding DMSO controls.

**Quantitative gene expression of pertinent CSC markers:**

Total RNA was extracted from blood of OSCC patients and healthy individuals using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. cDNA was synthesized from 1 μg RNA of each sample using High Capacity cDNA Reverse transcription kits (Applied Biosystems). Furthermore, real-time PCR reaction mix was prepared in a total volume of 20 μl comprising of 2X SYBR Fast qPCR Mastermix Universal (Kappa Biosystems), 200 nM of each primer, 2.5 mM of MgCl$_2$ and 1μl of cDNA. β-actin gene expression was measured as endogenous control. The amplification run was performed on the AriaMx Real-time PCR System (Agilent technologies) and the PCR reactions were subjected to 95°C for 3 min followed by 40 cycles of denaturation at 95 °C for 3 sec and annealing at 60 °C for 20 sec, for 40 cycles. The experiment was performed in triplicates and the relative mRNA levels were analyzed using the ddCt method after normalization with β-actin values.
Statistical Analysis:

Statistical analysis of gene expression profile with clinico-pathological parameters was done by Student’s t-test using SPSS version 13. Differences were expressed as mean ± SD and data was considered statistically significant when p-values was lower than 0.05.
RESULTS:

(A) Identification and characterization of circulating tumor stem like cells in peripheral blood of OSCC:

Circulating tumor stem like cells were isolated from heterogeneous subpopulation of OSCC patient derived peripheral blood samples using anti-CD44+ FITC labelled antibody by immuno-magnetic cell separation technique. Purity of the subpopulation was validated by gating the CD44+ sorted cells with CD44, CD24 and CD45 phenotypes using flow cytometry analysis. Based on the flow cytometry analysis of CD44+ cells, we identified two distinctive sub-populations: (i) CD44+ CD24+; (ii) CD44+ CD24- (Fig. 17). Our results depicted that out of the total parent population, 55% comprised of CD44+ pure population while 42% consisted of dual population containing CD44+ as well as CD24+ cells. However, absence of CD45+ cells in the sorted population confirmed the non-lymphocytic characteristic of these cancer stem like cell population (Fig. 21; Table10).

Figure 21: Flow cytometric validation on immuno-magnetically sorted CD44+ and CD44- subpopulation. Enriched CD44+ population was analyzed by flow cytometry and the image represents histograms of CD45, CD24 and CD44. Further, Q1 indicates the pure CD44+ population comprising of the majority of the total population while Q2 depicts the CD44+ CD24+ dual population. Image is representative of 3 independent experiments and presented graphically with mean ± SD.
### Table 10: Percentage of distinctive subpopulations quantified to assess the purity of CD44+ immuno-magnetically sorted cells using Flow Cytometric analysis

<table>
<thead>
<tr>
<th>Distinctive Subpopulation of CD44+ cells</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td>CD44+ (Pure)</td>
<td>55</td>
</tr>
<tr>
<td>CD24+ (Pure)</td>
<td>3</td>
</tr>
<tr>
<td>CD44+ CD24+ (Dual population)</td>
<td>42</td>
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</table>

We further subjected these sorted cell population to various cellular and molecular assays in order to understand their vital role as a circulatory prognostic markers and in assessing therapeutic efficacy in OSCC.

#### (B) Increased self-renewal potential of CD44+ cells:

Intrinsic sphere forming ability of CD44+ and CD44- cells was assessed to substantiate their CSC like phenotypic characteristic by seeding 1x 10^3 cells/well in ultra-low attachment 6 well plate. CD44+ subpopulation demonstrated enhanced colony forming ability with a significant increase in the number and size of spheres compared to their negative counterparts (Fig. 22).

![CD44+](image1.png) ![CD44-](image2.png)

**Figure 22: Self-renewal potential of immune-magnetically sorted CD44+ and CD44- subpopulation.** Representative images of tumor spheroids derived from immuno-magnetically sorted CD44+ and CD44- subpopulations at the end of 2 weeks.

Further, on assessing the proliferation rate, CD44+ cells demonstrated a colony forming efficiency of < 75% with the colonies exponentially increasing at regular intervals contradictory to their non stem cell-like counterparts, suggestive of the fact that this subpopulation possesses the intrinsic self renewal potential which is lacking in their normal counterparts. Collectively, these results clearly demonstrate
the colony initiating ability and self renewal potential of CD44+ cells - which are the two important characteristic hallmarks of CSCs.

(C) \textit{CD44+ cells demonstrated dose-dependent drug resistance:}

Intrinsic resistance to chemo- and radio-therapy is a unique characteristic feature of CSC population as these slow growing cells tend to frequently escape the conventional therapeutic regimen unlike the proliferating cells. Therefore, immuno-magnetically sorted CD44+ and CD44- cells were exposed to increasing concentrations of Cisplatin in order to determine the cytotoxic effect of this chemotherapeutic drug using MTT assay. CD44+ subpopulation demonstrated 74.3% viability compared to merely 32.2% viability of CD44- cells even at higher concentrations of cisplatin (1\textmu M; p-value <0.001) (Fig. 23; Table 11).

![Figure 23: Cytotoxic effect of Cisplatin on immune-magnetically sorted CD44+ and CD44- subpopulation.](image)

Image is representative of differences in Percent survival between CD44+ and CD44- cells. Data shown as mean ± SD and shows statistical significance at p<0.01 and lower concentration (0.125 \textmu g/ml) while p <0.001 significance was observed at higher concentrations (0.25, 0.5 and 1.0 \textmu g/ml).
Table 11: Survival rate (%) of CD44+ and CD44- subpopulations at various concentrations of Cisplatin (μg/ml)

<table>
<thead>
<tr>
<th>Conc of Cisplatin (μg/ml)</th>
<th>Survival rate of CD44+(%)</th>
<th>Survival rate of CD44-(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.125</td>
<td>92.5</td>
<td>79.6</td>
</tr>
<tr>
<td>0.25</td>
<td>87.1</td>
<td>64.3</td>
</tr>
<tr>
<td>0.5</td>
<td>80.1</td>
<td>48.7</td>
</tr>
<tr>
<td>1</td>
<td>74.3</td>
<td>32.2</td>
</tr>
</tbody>
</table>

Based on the percentage survival results, we could establish a probable association between the degree of drug resistance and the presence of CD44+ fraction; however further studies focusing on this aspect could enhance our understanding. Collectively, these results are suggestive of the fact that CD44+ cells were significantly chemo-resistant and showed dose dependent drug resistance to Cisplatin.

(D) Difference in expression patterns of pertinent csc markers in circulating CD44+ and CD44- subpopulation:

Quantitative gene expression analysis of CD44 isoforms (standard, v3 & v6) and stemness markers (Bmi1 & Nanog) was conducted in both CD44+ and CD44- subpopulations. Interestingly, CD44+ subpopulation demonstrated a significant upregulation in gene expression of CD44v3, CD44v6 and Nanog whereas CD44s showed significant down regulation in OSCC patients as compared to healthy control (p<0.001), depicting differential expression patterns of various isoforms in CD44+ sorted subpopulation. This prompted us to explore the difference in expression profile of CD44+ cells against CD44- population to identify the predominantly expressed CD44 variant and stemness marker which might be responsible for regulating the self renewal of these cells. Our results clearly demonstrated that CD44+ cells exhibited a significantly higher expression of CD44v6 and Nanog as compared to CD44- subpopulation indicating their role in conferring CD44+ cells its characteristic feature of self-renewal (Fig. 24; Table 12).
Figure 24: Gene Expression profiling of Pertinent CSC markers in CD44+ subpopulation. Representative graph indicates relative fold change of CSC markers - CD44s, CD44v3, CD44v6, Nanog and Bmi1 in CD44+ tumor spheres compared to normal controls and CD44- subpopulation. Data are expressed as mean + SD of 3 independent experiments with their p value <0.001.

Table 12: Fold change increase of CD44+ subpopulation compared to healthy control and CD44- cells assessed using Real Time PCR analysis.

<table>
<thead>
<tr>
<th></th>
<th>Fold change increase of CD44+ v/s Normal Control</th>
<th>Fold change increase of CD44+ cells v/s CD44- cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD44s</td>
<td>0.5586</td>
<td>1</td>
</tr>
<tr>
<td>CD44v3</td>
<td>3.4105</td>
<td>0.9930</td>
</tr>
<tr>
<td>CD44v6</td>
<td>54.5686</td>
<td>29.8570</td>
</tr>
<tr>
<td>Nanog</td>
<td>15.2422</td>
<td>11.1579</td>
</tr>
<tr>
<td>Bmi1</td>
<td>1.7052</td>
<td>1.6021</td>
</tr>
</tbody>
</table>

Note: Bold values depict significant increase in the fold change of CD44+ cells compared to control while the underlined values depict significant increase in fold change of CD44+ cells compared to CD44- cells.

Therefore, it was indispensable to further assess the gene expression patterns of these two markers in OSCC patients and comprehend its clinical relevance.
(E) Association of increased expression patterns of CD44s, CD44v6 and Nanog with anatomic location, loco-regional aggressiveness and recurrence in OSCC:

To further explore the role of CSC markers - CD44s, CD44v6 and Nanog in OSCC, we analyzed the gene expression levels and their association with the various clinico-pathological characteristics of oral carcinoma patients. Our results demonstrated an increase in expression patterns of CD44v6 and Nanog (p<0.001) and a simultaneous decrease in expression levels of CD44s in OSCC patients as compared to healthy controls (Fig. 25). However, significantly higher expression of CD44v6 and Nanog were distinctly found in late stage and loco-regionally aggressive patients compared to early stage and non-aggressive OSCC counterparts. These findings were consistent with the results of gene expression analysis in CD44+ subpopulation and justified the in-vitro observation of CD44+ cells demonstrating reduced efficacy towards conventional cisplatin therapy. To further substantiate whether gene expression of these CSC markers have any association with disease progression, we analyzed the correlation between clinico-pathological conditions of oral carcinoma patients and expression of CD44s, CD44v6 and Nanog.

![Figure 25: Gene Expression profiling of Pertinent CSC markers in OSCC patients.](image)

**Figure 25:** Gene Expression profiling of Pertinent CSC markers in OSCC patients. Scatter plot depicts relative quantification of CSC markers - CD44s, CD44v6 and Nanog in 30 OSCC patients with their p values indicated in the graphs as ***p<0.001.

Buccal mucosa carcinoma patients demonstrated a significantly higher gene expression of CD44v6 and Nanog compared to tongue cancer patients (p<0.05), prompting towards the possibility that buccal mucosa as a subsite might have more
aggressive behaviour that would be difficult to eliminate with the conventional chemo-therapeutic regimen (Fig. 26).

![Graph showing differential expression of CSC markers CD44v6 and Nanog in diverse anatomic locations of tongue and buccal mucosa respectively with their p values indicated in the graphs as *p<0.05.]

Figure 26: Differential expression of CSC markers CD44v6 and Nanog in diverse anatomic locations of tongue and buccal mucosa respectively with their p values indicated in the graphs as *p<0.05.

Apart from considerable difference found in gene expression patterns based on different anatomical subsites, an increasing trend of CD44v6 and Nanog expression was observed in habituated patients compared to non-habituated patients; however, these results were not statistically significant attributed to the lower sample size of non-habituated patients. Since the tumor stage reflects the invasive nature of the malignancy, we assessed the expression of circulating tumor stem like cells with TNM stage of OSCC patients. Significant association was observed between expression pattern of CD44v6 and patients with lymph node metastasis with a considerable increase in expression levels of patients with higher stages (N2b) compared to early stages (N0/N1; p<0.01) (Fig. 27A). Moreover, a noteworthy observation was noted with significantly increased expression of both CD44v6 and Nanog in recurrent OSCC cases compared to non-recurrent cases, indicating a possible role of these markers as secondary malignancy risk predictors (p<0.002) (Fig. 27B).
Figure 27: Differential expression of CSC markers with various clinico-pathological conditions. (A) Representative box plots depicts differences in relative expression patterns of CD44v6 marker in primary malignant conditions compared to loco-regionally aggressive OSCC patients (p<0.01). (B) Image is representative of relative expression of CD44v6 and Nanog in recurrent cases compared to non-recurrent patients respectively. The data is expressed as mean ± SD with their p values indicated in the graphs as **p<0.01 and ***p<0.001.

Collectively, these results clearly indicate towards an inevitable role of CD44v6 and Nanog in self-renewal and chemo-resistance which is substantiated by expression profile in patients.
DISCUSSION:

OSCC is a complex disease with immense tumoral heterogeneity and genomic complexity that can be characterised at the molecular and cellular level by acquiring tumor tissue at the time of diagnosis. Difficulty in accessibility of tumor in deeper sites, inadequacy of tissue, increasing the possibility of metastatic spread via invasive techniques, requirement of repetitive biopsies to monitor genetic mutational changes, discomfort and inherent clinical risk to the patient and C > T transitions due to formalin fixation resulting in false positive results are some of the major limitations of tissue biopsy hindering the development of a reliable prognostic marker, scrutinizing treatment response and facilitating personalized treatment decisions. [278, 286].

CTCs, a subpopulation of quiescent cells that reflect mutational landscape and frequent genotypic alterations occurring in the primary tumor, have demonstrated immense potential as non-invasive indicators of disease progression (liquid biopsies), constitutional basis for tumor staging, multifunctional biomarker to assess the treatment response and potential markers for targeted therapy [288]. Inspite of its undisputable implications, there is lack of a comprehensive study emphasizing on identification of a multifunctional CTC based biomarker, optimizing the isolation & characterisation techniques and investigating their significance in terms of clinical relevance in OSCC. Recent evidences detected CTCs in almost all HNSCC cell lines (FaDu) using FDA approved EpCAM marker based approach; however this rare population was not detectable in more than 50% of metastatic and aggressive patients of HNSCC which was in corroboration with the findings of other studies [191, 289].

One of the major rationale of these failures particularly in the advanced disease patients undergoing EMT, could be the exclusion of non-EpCAM expressing CTCs. Additionally, relying on epithelial features could lead to the possibility of false positive results as the presence of these circulating epithelial cells are prevalent in non-malignant conditions such as inflammatory colon [173]. Thus, need of the hour is to identify a marker that could address this unsolved paradox by understanding the characteristics and mutational alterations of CTCs in order to enhance the current modalities of identifying CTCs as non-invasive prognostic markers of OSCC. Therefore, in this study we identified CD44+ CD24- CD45- CSC like subpopulation from mononuclear cells of oral cancer patients in order to elucidate the phenotypic classification, molecular characterisation and clinical relevance of these circulating cancer stem like cells.
In view of the fact that EMT is attributed to CD24 depletion in certain malignancies including OSCC, we considered CD44+ CD24- population over CD44+ CD24+ population for our study [285]. The identification of CD44+ subpopulation in circulation of OSCC patients is speculated to be a resultant repercussion of CD44 extracellular domain shedding, comprising of variant exons and binding site of this hyaluranon receptor, thus having a plausible role in progression of this malignancy [290]. Further, our cellular assay results suggested that CD44+ cells formed significantly larger spheres representing properties such as self-renewal and tumor initiating potential which are defining hallmarks of CSCs. These characteristics might facilitate the circulatory tumor stem like cells in sustaining the apoptotic mechanism in circulation and plausibly form secondary tumors on finding a suitable niche. Collectively, these findings suggest that CD44+ cells could be a suitable CTC marker over epithelial based biomarkers to monitor disease progression in post EMT OSCC patients. Moreover, results of the cytotoxicity assay exhibited 70% viability of CD44+ cells at the highest concentration of Cisplatin, indicating that these cells have the potential to evade the conventional chemotherapeutic regimen leading to dose dependent drug resistance in patients which is in corroboration with previous reports of Shuyang et al [77]. These findings suggest that presence of CTCs with stem like property would reduce the effectiveness of this drug leading to low therapeutic response, recurrence and relapse in OSCC patients.

This distinct phenotypic behavioural pattern of circulating tumor stem like cells prompted us to conduct the molecular characterisation of these CD44+/− subpopulations with the hope to identify pertinent CSC markers responsible for maintaining the stemness characteristic of this population. Although, all the CSC markers assessed (CD44v3, CD44v6 and Nanog) showed increased expression in CD44+ cells compared to their normal counterparts; a significant upregulation of CD44v6 and Nanog markers was observed in CD44+ cells compared to their non-stem cell like population indicative of their undisputable role in governing the self-renewal and conferring stemness property. Taking into consideration that recent evidence associated the presence of CTCs with tumor staging, disease progression and poor prognosis; it is becoming increasingly evident to understand the expression pattern of these disseminated tumor population clinically [278]. On evaluating the gene expression profile of CD44v6 and Nanog clinically, we observed a significant upregulation of these markers in almost all oral cancer patients which has been
observed in primary tumor samples of the same set of OSCC patients in our previous study [291]. Moreover, our results are in corroboration with the reports of Zhou et al and Sodja et al in ovarian and lung cancer; however our findings contradict the results of Song et al observing no significant difference in CD44v6 expression patterns in peripheral blood of cancer patients compared to control [291-293]. To the best of our knowledge this is one of the preliminary studies signifying the importance of CD44v6 and Nanog transcript level expression patterns in CTCs of OSCC as probable prognostic markers and suitable targets for personalised therapy.

Further convoluted statistical analysis demonstrated a strong association of CD44v6 expression levels with different clinicopathological conditions - anatomic locations, loco-regional aggressiveness and relapse. The diverse expression pattern of CD44v6 in circulating tumor stem like cells of patients with different subsites of OSCC is consistent with our previous findings wherein a similar behavioural pattern was observed in primary tumor of the same set of OSCC patients [291]. Since, shedding of CTCs occurs from the tumor bulk, their enhanced expression profiles at the tissue level make them inherently more prone to express CD44v6 marker at the circulatory level. Moreover, increased expression of this CSC marker in buccal mucosa at the tumor levels are indicative of the fact that different subsites of OSCC behave as independent entities and function with diverse context dependent role which could be attributed to their different embryogenic origins as these markers have a role in maintenance of pluripotency in ESCs. Predominant CD44v6 expression in loco-regional aggressiveness of the disease is suggestive of its prognostic and clinical utility to predict the early onset of metastases in OSCC patients. Additionally, our results depicted a strong association of CD44v6 and Nanog with recurrent OSCC patients, prompting towards no significant effect of chemo-radio therapy on circulating tumor stem like cell population which is consistent with our in-vitro findings and might have a vital role in endowing this malignancy with an aggressive and invasive behaviour.
KEY FINDINGS:

- CD44, pertinent CSC marker, might be enhancing the aggressive, invasive and metastatic behavior of the malignancy via shedding of the extracellular domain of CD44 molecule from the tumor bulk into the circulation of patients.

- The stem like property of CD44 cells confers it with the unique ability of self-renewal and intrinsic resistance to chemotherapeutic drugs, facilitating this circulating tumor stem like cell population to survive the apoptotic mechanisms in circulation and escape drug therapy.

- CD44v6 could be utilized as potential prognostic marker to monitor the disease progression, locoregional metastases and therapeutic response along with its stemness marker.

- Nanog acts as a close ally and adds to the functional relevance of this CSC marker.

Published in and as: