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# Cancer Stem Cells and Somatic Stem Cells as Potential New Drug Targets, and Prognosis Markers, and Therapy Efficacy Predictors in Breast Cancer Treatment

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**Abstract:** Breast cancer (BC) is one of the leading causes of cancer death in women. Thus, the search for drug targets, markers of disease prognosis, and more efficient treatment options is relevant. We have conducted a pilot study including patients with luminal B stage breast cancer IIA-IIIB (T1-3N0-3M0). The control group consisted of healthy women. Presence and frequency of various populations of cancer stem cells (CSC) and somatic stem cells were assessed in the blood, breast tumor tissue, and normal breast tissue. Our results suggest that patients with BC can be divided into two distinct groups based on the frequency of aldehyde dehydrogenase positive cells (ALDH1+ cells) in the blood ($ALDH1^{hi}$ and $ALDH1^{low}$). In the $ALDH1^{hi}$ cells group, the tumor is dominated by epithelial tumor cells $CD44^{+}CD24^{low}$, $CD326^{+}CD44^{+}CD24^{-}$ and $CD326^{-}CD49f^{+}$, in the $ALDH1^{low}$ cells group, the CSCs of mesenchymal origin ($CD44^{+}CD24^{-}$) and epithelial tumor cells ($CD227^{+}CD44^{+}CD24^{-}$ and $CD44^{+}CD24^{-}CD49f^{+}$). In vitro CSCs of the $ALDH1^{low}$ cells group expressing CD326 showed high resistance to cytostatics, $CD227^{+}$ CSCs of the $ALDH1^{hi}$ cells group are sensitive to cytostatics. Populations of epithelial progenitor cells of healthy mammary gland were revealed in normal breast tissue of patients with BC from both groups. The cells were associated with a positive effect of chemotherapy and remission in BC patients.

**Keywords:** breast cancer, ALDH1, cancer stem cells, somatic stem cells, biomarkers

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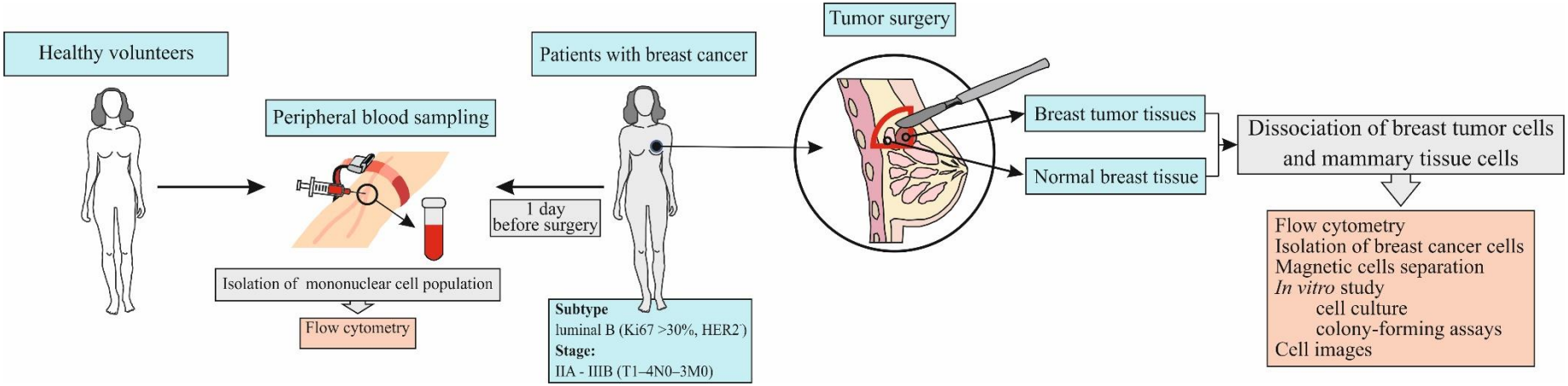
# Results and Discussion

The experimental design of investigation of patients and volunteers




The pilot study included 12 patients with IIA - IIIB (T1–4N0–3M0) breast cancer of the luminal B with average age of 47,4 ± 0,8 years. The luminal B subtype of breast cancer was defined as $ER^+$, $PR^+$ or -, Ki67 >30%, and all patients with the luminal B subtype were HER2-negative. Histological diagnosis was confirmed for all samples.

Blood samples were obtained from patients one day before surgery. Breast tumor and normal breast tissue were obtained from patients on the day of surgery.

Blood samples from 10 healthy women of similar age were used as control.

The study included patients who received treatment at the Cancer Research Institute of Tomsk NRMC (Tomsk, Russia).

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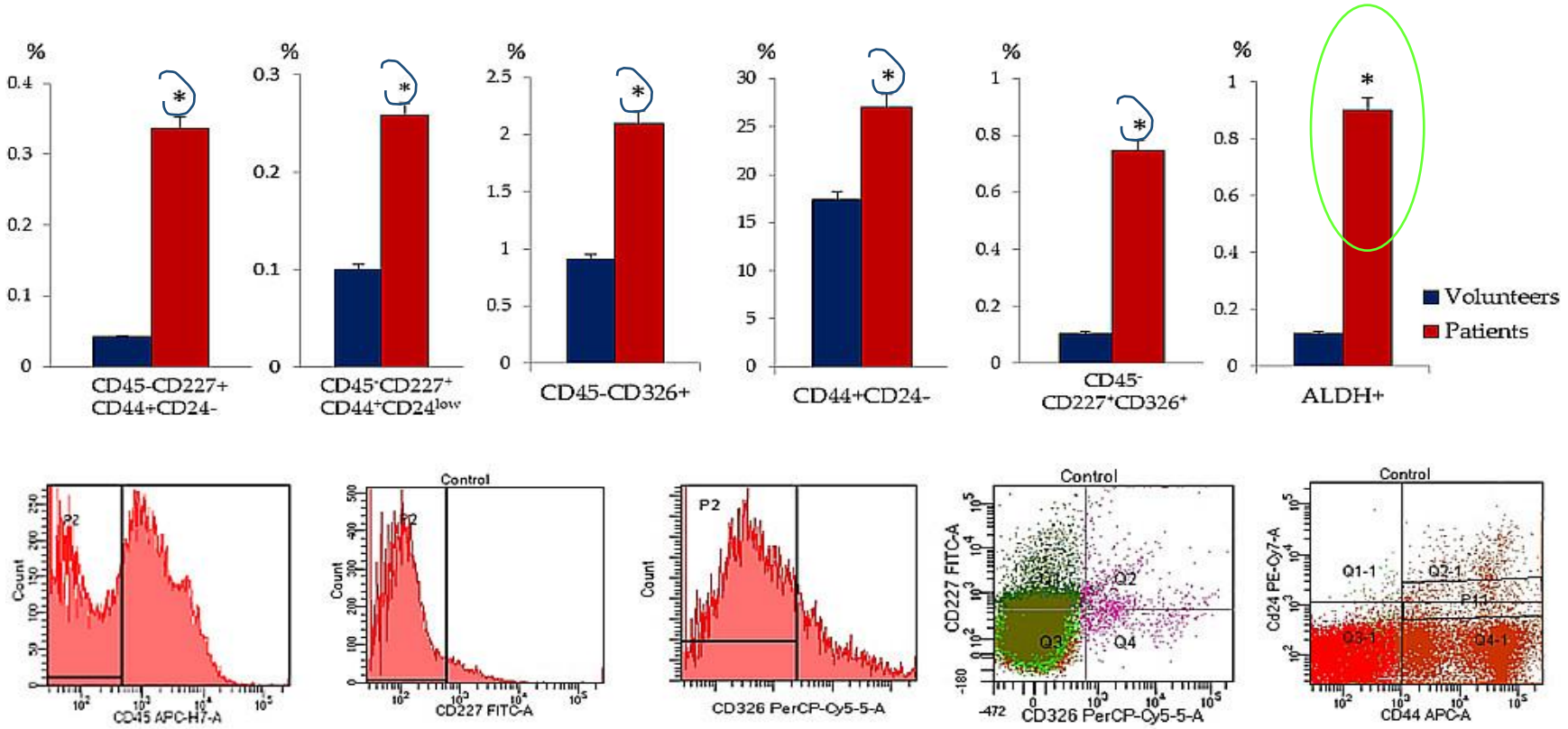
# Results and Discussion

The level of epithelial tumor cells and tumor stem cells blood of patients with breast cancer and healthy volunteers




* - differences are significant in comparison with the healthy volunteers (P<0.05).

In blood samples from patients with breast cancer the number of tumor cells with overexpression of CD227 and CD326 was increased, as well as cancer stem cells of mesenchymal origin ($CD44^+CD24^-$) relative to healthy volunteers. In breast cancer an increase in the number of ALDH1+ cells circulating in the blood was observed.

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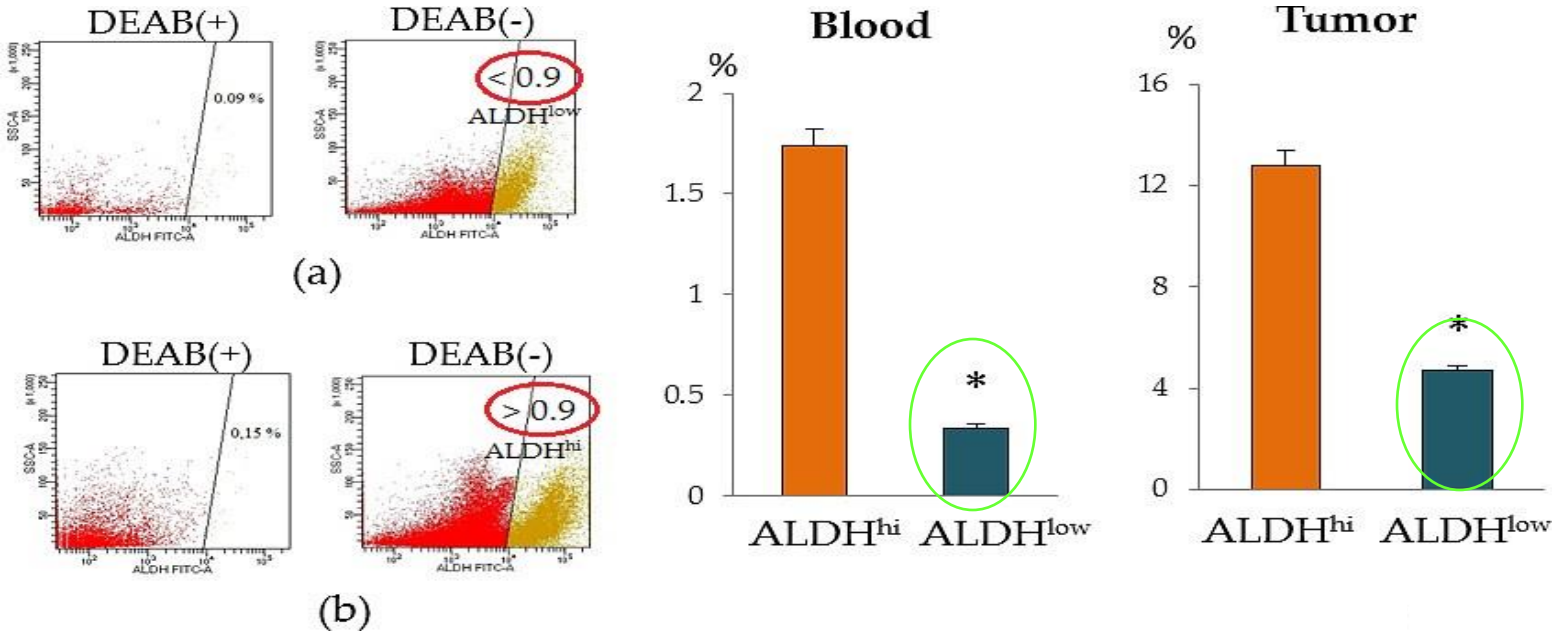
# Results and Discussion

The level of ALDH1+ cells in the blood and tumor of patients with breast cancer




The patients with breast cancer were divided into two distinct groups based on the level of aldehyde dehydrogenase positive cells (ALDH1+ cells) in the blood:

a) The BC patients with ≤0.9 % of all isolated mononuclear cells - $ALDH1^{low}$
b) The BC patients with ≥0.9 % of all isolated mononuclear cells - $ALDH1^{hi}$

The level of $ALDH1^{+}$ cells in the tumor of patients of $ALDH1^{hi}$ cells group was also higher than that of patients of $ALDH1^{low}$ cells group.

* - differences are significant in comparison with the $ALDH1^{hi}$ group ($P<0.05$).

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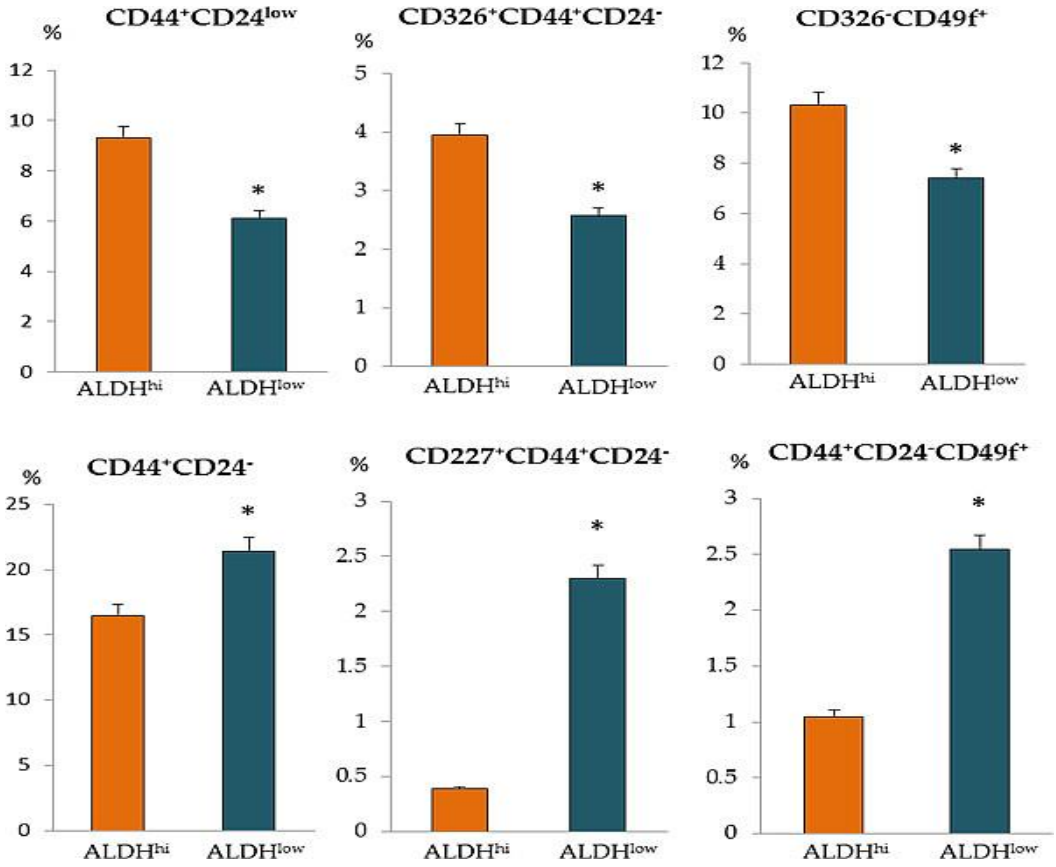
# Results and Discussion

Characterization of epithelial tumor cells and tumor stem cells population isolated from tumor of patients with breast cancer




The ALDH1$^{hi}$ cells group showed an increased number of epithelial tumor cells CD44$^{+}$CD24$^{low}$, CD326$^{+}$CD44$^{+}$CD24$^{-}$, and CD326$^{-}$CD49f$^{+}$.

The content of cancer stem cells of mesenchymal origin (CD44$^{+}$CD24$^{-}$) and epithelial tumor cells (CD227$^{+}$CD44$^{+}$CD24$^{-}$ and CD44$^{+}$CD24$^{-}$CD49f$^{+}$) in the tumor prevailed.

* - differences are significant in comparison with the ALDH1$^{hi}$ group ($P<0.05$).

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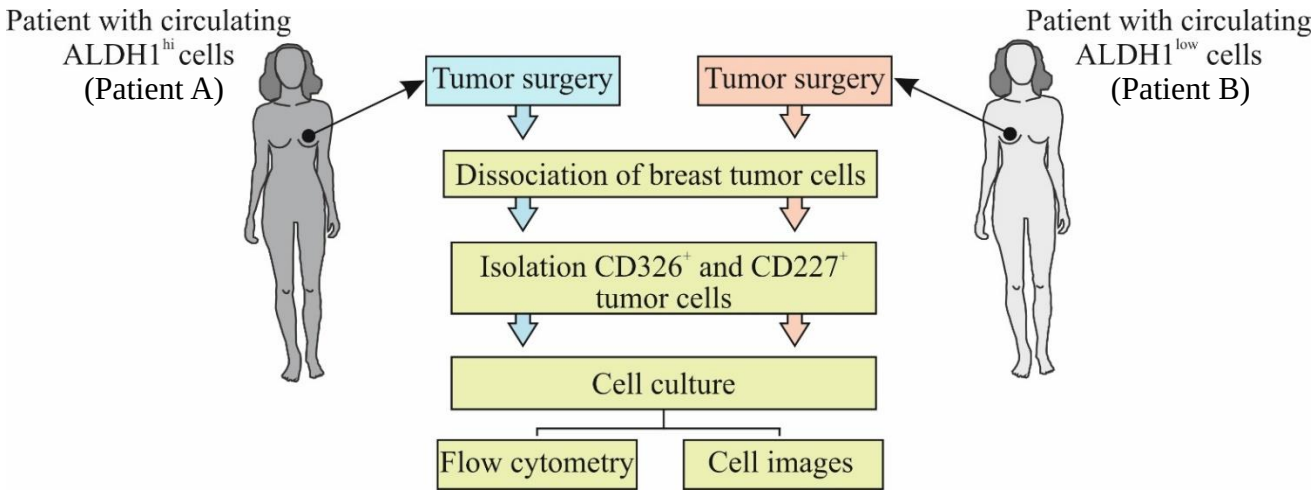
# Results and Discussion

*In vitro* tumor study




The sorted cells were seeded and cultured in the presence MammoCult™ supplemented with 0.48 µg/mL freshly dissolved hydrocortisone and 4 µg/mL heparin and 10 ng/mL IL-6 to induce greater numbers of mammospheres and tumorspheres, and the cultures were maintained for an additional 7 to 10 days.

The $CD227^+$ and $CD326^+$ sorted cell populations were cultured in the presence of 10 ng/mL cytostatics (doxorubicin+docetaxel+cyclophosphamide), after 2 h culture we evaluated apoptosis using flow cytometry and image processing of each well with Cytation™ 3.

* Informed consent was obtained from all individual participants included in the study

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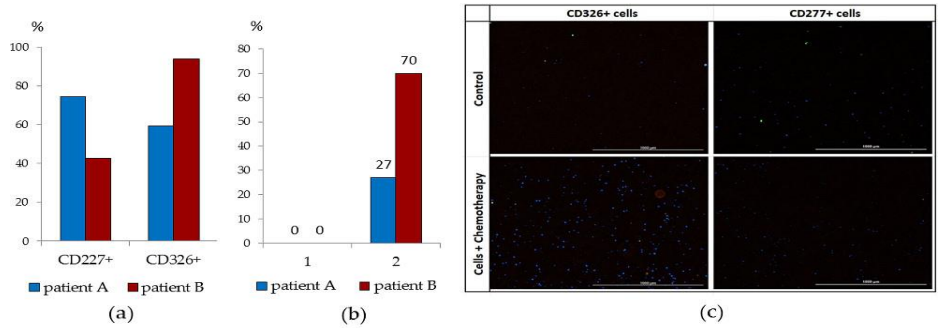
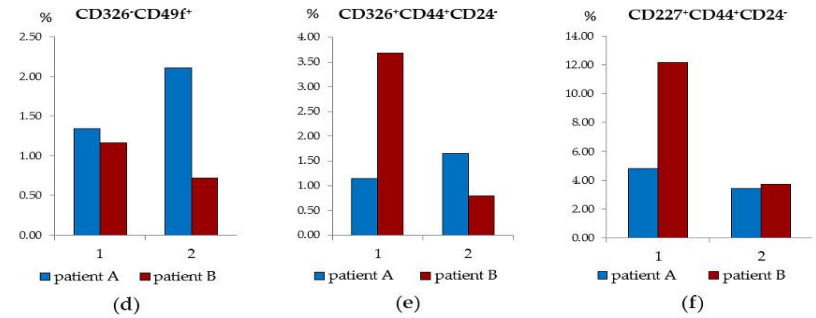
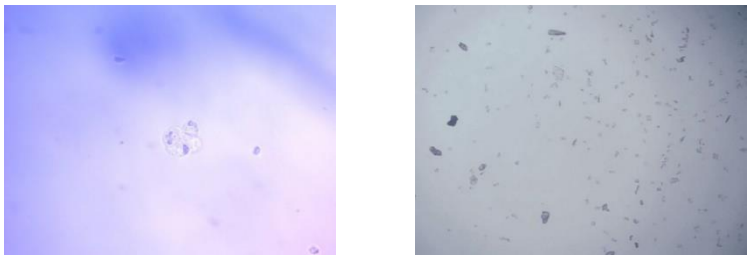
# Results and Discussion

## The tumor cell activity from patients A and B *in vitro*





(g)


(h)

The culture of CD326⁺ cells of patient A withstood three passages and by the end of the cultivation cycle its cell mass significantly increased, under the influence of IL-6 the activity of mammosphere formation increased. These culture parameters of patient A were superior to those of patient B.

In patient B, the culture of CD227⁺cells was characterized by the absence of cells in apoptosis, insignificant clonal activity (mammosphere) and an increase in cell mass during the cultivation cycle.

In culture of CD227⁺ cells of patient B, IL-6 increased the activity of mammosphere formation. After co-cultivation with cytostatics, the number of CD227⁺ cells in apoptosis was 70% of the total.

**(a)** The content of tumor stem cells in the CD227+ and CD326+ cell enriched environment after a cycle of cultivation and IL-6 treatment; **(b)** The count of tumor cells with apoptosis after a cycle of cultivation without cytostatic and with cytostatics; **(c)** Determination of the percent of cells in apoptosis (the ratio of cells counted in green and red channel to total cells counted using blue (DAPI) channel; **(d-e)** The count of tumor cells after a cycle of cultivation without cytostatic and with cytostatics (doxorubicin+docetaxel+cyclophosphamide) of patient A and patient B. (g-h) Nonadherent mammospheres of CD326⁺ cancer cell cultures (A patient) and CD326⁺without mammosphere formation (B patient).

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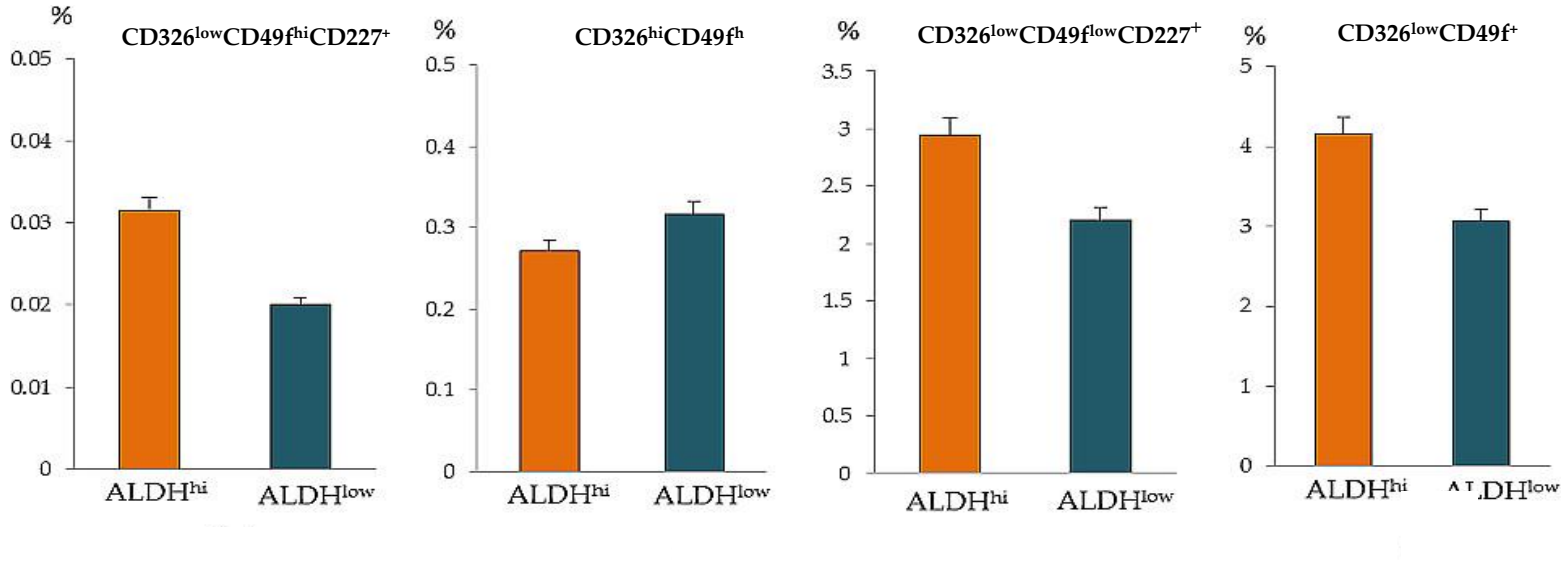
# Results and Discussion

Characterization of bipotent precursors of breast cells isolated from the breast tissue adjacent to the tumor of patients with breast cancer




In the breast tissue adjacent to the tumor (normal tissue) of patients with breast cancer, different populations of epithelial progenitor cells of healthy mammary gland were revealed. Significant differences between the patients of the ALDH1$^{hi}$ and ALDH1$^{low}$ groups did not find.

In all groups, cells were associated with a positive effect of chemotherapy and remission of patients with breast cancer. Perhaps, for patients with stable remission, it is necessary to carry out measures aimed at the epithelium and endothelium regeneration of the mammary gland, which, in our opinion, will make it impossible for the disease to recur.

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# Conclusions

Patients with IIA-IIIB (T1-4N0-3M0) breast cancer are divided into a group with a significant number of ALDH1$^{+}$ cells and a group with a small number of ALDH1$^{+}$ cells in the blood and tumors.

The composition of cancer stem cells and their activity in patients of the ALDH1$^{hi}$ cells group and the ALDH1$^{low}$ cell group differ. ALDH1 expression level and ratio of tumor cells CD44$^{+}$CD24$^{low}$, CD326$^{+}$CD44$^{+}$CD24$^{-}$, CD326$^{-}$CD49f$^{+}$, cancer stem cells of mesenchymal origin (CD44$^{+}$CD24$^{-}$) and epithelial tumor cells (CD227$^{+}$CD44$^{+}$CD24$^{-}$ and CD44$^{+}$CD24$^{-}$CD49f$^{+}$) in tumors can act as personalized diagnostic markers, predictors of complications and the effectiveness of breast cancer treatment in further research.

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## Ethics statement

Informed consent was obtained from all individual participants included in the study. All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## Conflicts of interest

The authors declare no conflict of interest.

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# Thank you for attention!

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