Chapter 8

CONCLUSION AND RECOMMENDATIONS

The role of tumor-initiating cells (popularly known as cancer stem cells) in tumor development and availability of these markers promises a new arena in understanding their role in developing novel targeted molecules. It is important to identify and understand the relevance of cancer stem cells (CSC) in tumor development and to design appropriate strategies for CSCs elimination, which is crucial to future cancer prevention and treatment.

In the present study CD44 was more extensively and strongly expressed in OSMF cases than ALDH1A1. In contrast, all numbers of ALDH1+ cases also expressed for CD44 marker. Numbers of ALDH1+ OSMF cases and CD44+/ALDH1+ OSMF cases were the same amount. It can be suggested that every ALDH1+OSMF cases may express with CD44 enzyme in OSMF. Both proteins were prominent in male patients and in ≤30 years of age range. Protein expressions were more prominent in duration of signs and symptoms ≤6 months. As the pathological variance, both proteins showed higher expression in association with lack of lymphocytic infiltration in epithelial connective tissue junction in OSMF cases. Lymphocytic infiltration was favored for negative expression of both markers. Hyalinization showed no significant effect on expression pattern. Fibroblasts gave rise to positivity of CSCs. Less blood vessel was favored for decreased expression of CSCs. In muscle atrophy cases, amounts of CD44+/ALDH1A1+ cells are prominent.

CD44 and ALDH1A1 were detected nearly the same number in OSCC cases. Male was predominant than female and age range that markers were

prominently detected in OSCC was in 31-60 years of age and >6 months duration of signs and symptoms. ALDH1A1+ cells were high in 20-50% keratinization and small groups or cords of invasive front. Moderate amount of nuclear polymorphism was directly associated with CD44 and ALDH1 positivity. CD44 was higher in cases of 2-3 mitosis. In OSCC, lymphoplasmacytic infiltration is inversely related with CD44+/ALDH1A1+ cells, that is, in marked lymphoplasmacytic infiltration, expression of CD44 and ALDH1A1 was low and in slight lymphoplasmacytic infiltration, expression rate of markers was high. In Bryne's classification, positivity of CD44 marker tends to be high in Grade III OSCC stage and ALDH1 marker was relatively high in Grade I and II OSCC stages.

Generally, expression rate of CD44 and ALDH1 protein was higher in OSMF than in OSCC. Especially, CD44 showed higher expression in OSMF (80%) than in OSCC (58.82%). In male, expression rate was higher in OSMF, but in female, it was directed to OSCC. OSMF cases were prominently seen <30 years of age, OSCC cases were evident in 31-60 years of age groups and its highest rate was seen in >60 years of age groups. Expression rates of both markers in two entities were directly proportionate to duration of disease signs and symptoms.

Both markers showed expression in tumor portions and surrounding non-tumor portions in OSCC as well as non-tumor epithelium of OSMF. These findings were consistent with the concept of field cancerization.

In conclusion, CD44 and ALDH1 expression shows a more distinct distribution pattern in oral premalignant lesion, OSMF. Thus, according to this finding, ALDH1 and CD44-positive cells in epithelium of OSMF and in adjacent non-tumor epithelium as well as tumor portion of OSCC suggests that changes were already underway, as these enzymes tend to be present in cells with a high

tumorigenic potential. CD44 and ALDH1 appear to be important factors in carcinogenesis and tumor progression in OSCC and an important role in pathogenesis of OSMF. Positivity of both markers was directly correlated with Bryne's tumor grade and inversely correlated with tumor-host immune reaction. CD44 and ALDH1 may be expressed in the CSCs of most examined tumors and OSMF. However, immunohistochemically technique here employed was not appropriate to identify cancer stem cells in HNSCCs and these markers are not sufficient to precisely isolate the CSC subpopulation from the tumor bulk. Further protein markers such as CD133, CD24, CD31 and c-met are needed to precisely define the CSC subpopulations in OSMF and OSCC.

REFERENCES

Abdul Majeed, A. A., Dalley, A. J., & Farah, C. S. (2013). Putative cancer stem cell marker expression in oral epithelial dysplasia and squamous cell carcinoma. *Journal of Oral Pathology & Medicine*, *42*(10), 755–760. http://doi.org/10.1111/jop.12073

Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., Clarke, M.F., (2003). Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA*. 100:pp. 3983–8.

Angadi, P. V, Kale, A. D., & Hallikerimath, S. (2011). Evaluation of myofibroblasts in oral submucous fibrosis: correlation with disease severity. *Journal of Oral Pathology & Medicine : Official Publication of the International*

Association of Oral Pathologists and the American Academy of Oral Pathology, *40*(3), 208–213. http://doi.org/10.1111/j.1600-0714.2010.00995.x

Angadi, P. V., & Rao, S. S. (2011). Areca nut in pathogenesis of oral submucous fibrosis: Revisited. *Oral and Maxillofacial Surgery*. http://doi.org/10.1007/s10006-010-0219-8

Angadi, P. V., & Rekha, K. P. (2011). Oral submucous fibrosis: A clinicopathologic review of 205 cases in Indians. *Oral and MaxillofacialSurgery*,*15*(1),15–19.http://doi.org/10.1007/s10006-010-0225-x

Angadi, P.V., Krishnapillai, R., (2012). Evaluation of PTEN Immunoexpression in Oral Submucous Fibrosis: Role in Pathogenesis and Malignant Transformation. *Head and Neck Pathol*. 6:pp. 314–321.

Anneroth, G., Batsakis, J., Luna, M., (1987). Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. *Scand J Dent Res*. 95(3): 229-49.

Arakeri, G., & Brennan, P. a. (2013). Oral submucous fibrosis: An overview of the aetiology, pathogenesis, classification, and principles of management. *British Journal of Oral and MaxillofacialSurgery*,*51*(7),587–593. http://doi.org/10.1016/j.bjoms.2012.08.014

Bahar, R., Kunishi, M., Kayada, Y., & Yoshiga, K. (1997). CD44 variant 6 (CD44v6) expression as a progression marker in benign, premalignant and malignant oral epithelial tissues. *International Journal of Oral and Maxillofacial Surgery*, *26*(6), 443–446. http://doi.org/10.1016/S0901-5027(97)80010-0

Beachy, P. A., Karhadkar, S. S., & Berman, D. M. (2004). Tissue repair and stem cell renewal in carcinogenesis. *Nature*, *432*(7015), 324–331. http://doi.org/10.1038/nature03100

Beck, B., & Blanpain, C. (2013). Unravelling cancer stem cell potential.*NatureReviews.Cancer*,*13*(10),727–38. http://doi.org/10.1038/nrc3597

Bhaijee, F., Pepper, D. J., Pitman, K. T., & Bell, D. (2012). Cancer stem cells in head and neck squamous cell carcinoma: A review of current knowledge and future applications. *Head & Neck*, *34*(6), 894–899. http://doi.org/10.1002/hed.21801

Bishen, K. A., Radhakrishnan, R., & Satyamoorthy, K. (2008). The role of basic fibroblast growth factor in oral submucous fibrosis pathogenesis. *Journal of Oral Pathology & Medicine : Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *37*(7), 402–411. http://doi.org/10.1111/j.1600-0714.2008.00649.x

Bjerkvig, R., Tysnes, B., & Aboody, K. (2005). The origin of the cancer stem cell: current controversies and new insights. *Nature ReviewsCancer*,*5*(November),899–904. http://doi.org/10.1038/nrc1740

Borovski, T., De Sousa E Melo, F., Vermeulen, L., & Medema, J. P. (2011). Cancer stem cell niche: The place to be. *Cancer Research*. http://doi.org/10.1158/0008-5472.CAN-10-3220

Brack, A. S., Conboy, M. J., Roy, S., Lee, M., Kuo, C. J., Keller, C., & Rando, T. A. (2007). Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science (New York,N.Y.)*,*317*(5839),807–810. http://doi.org/10.1126/science.1144090

Broders, A.C., (1920). Squamous-cell epithelioma of the lip. *J Am Med Assoc* 74: 656-664

Bryne, M., Jenssen, N., and Boysen, M., (1995). Histological grading in the deep invasive front of TI and T2 glottic squamous cell carcinomas has high prognostic value. *Virchows Arch* 427: 277-281

Bryne, M., Koppang, H.S., Lilleng, R., and Kjaerheim, A., (1992). Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. *J Pathol* 166: 375-381

Bryne, M., Koppang, H.S., Lilleng, R., Stene, T., Bang, G., and Dabelsteen, E., (1989). New malignancy grading is a better prognostic indicator than Broders' grading in oral squamous cell carcinomas. *J Oral Pathol Med* 18: 432-437

Cabarcas, S. M., Mathews, L. A., & Farrar, W. L. (2011). The cancer stem cell niche-there goes the neighborhood? *International Journal of Cancer*, *129*(10), 2315–2327. http://doi.org/10.1002/ijc.26312

Chen, Y.-C., Chen, Y.-W., Hsu, H.-S., Tseng, L.-M., Huang, P.-I., Lu, K.-H., Lo, W.-L. (2009). Aldehyde dehydrogenase 1 is a putative marker for cancer stem cells in head and neck squamous cancer. *Biochemical and Biophysical Research Communications*, *385*(3), 307–313. http://doi.org/10.1016/j.bbrc.2009.05.048

Chen, Z. G., Nannapaneni, S., Muller, S., Saba, N. F., Tighiouart, M., Pan, L., & Shin, D. M. (2010). Evaluation of ALDH1-A1 as a biomarker for metastasis of head and neck cancer. *Journal of Clinical Oncology*, *28*(15). http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L70260139\nhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=0732183X&id=doi:&atitle=Evaluation+of+ALDH1A1+as+a+biomarker+for+metastasis+of+head+and+neck+cancer&stitle=J.+Clin.+Oncol

Chiba, I. (2001). Prevention of betel quid chewers' oral cancer in the asian-pacific area. *Asian Pacific Journal of Cancer Prevention*.

Cho, R. W., & Clarke, M. F. (2008). Recent advances in cancer stem cells. *Current Opinion in Genetics & Development*, *18*(1), 48–53. http://doi.org/10.1016/j.gde.2008.01.017

Cichy, J., & Puré, E. (2003). The liberation of CD44. *Journal of Cell Biology*. http://doi.org/10.1083/jcb.200302098

Clarke, M.F., Dick, J.E., Dirks, P.B., *et al*., (2006). Cancer stem cells–perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res*. 66:pp. 9339-44.

Collins, A.T., Berry, P.A., Hyde, C., Stower, M.J., Maitland, N.J., (2005). Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res*. 65:pp. 10946–51.

Cuff, C. A., Kothapalli, D., Azonobi, I., Chun, S., Zhang, Y., Belkin, R., Puré, E. (2001). The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *Journal of Clinical Investigation,108*(7),1031–1040. http://doi.org/10.1172/JCI200112455

Dalerba, P., Cho, R. W., & Clarke, M. F. (2007). Cancer stem cells: models and concepts. *Annual Review of Medicine*, *58*, 267–284. http://doi.org/10.1146/annurev.med.58.062105.204854

Dontu, G. (2008). Breast cancer stem cell markers - the rocky road to clinical applications. *Breast Cancer Research : BCR*. http://doi.org/10.1186/bcr2130

Fábián, Á., Vereb, G., & Szöllosi, J. (2013). The hitchhikers guide to cancer stem cell theory: Markers, pathways and therapy. *Cytometry Part A*, *83A* (1), 62–71. http://doi.org/10.1002/cyto.a.22206

Garcion, E., Naveilhan, P., Berger, F., & Wion, D. (2009). Cancer stem cells: Beyond Koch's postulates. *Cancer Letters*. http://doi.org/10.1016/j.canlet.2008.09.006

Gil, J., Stembalska, A., Pesz, K. A., & Sasiadek, M. M. (2008). Cancer stem cells: the theory and perspectives in cancer therapy. *Journal of Applied Genetics*, *49*(2), 193–199. http://doi.org/10.1007/BF03195612

Ginos, M.A., Page, G.P., Michalowicz, B.S., *et al*., (2004). Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. *Cancer Res*. 64:pp. 55–63.

Goodison, S., Urquidi, V., & Tarin, D. (1999). CD44 cell adhesion molecules. *Molecular Pathology : MP*, *52*(4), 189–196. http://doi.org/10.1136/mp.52.4.189

Goodison, S., Urquidi, V., & Tarin, D. (1999). CD44 cell adhesion molecules. *Molecular Pathology : MP*, *52*(4), 189–196. http://doi.org/10.1136/mp.52.4.189

Gupta, P. C., & Ray, C. S. (2004). Epidemiology of betel quid usage. In *Annals of the Academy of Medicine Singapore* (Vol. 33).

Harper, L. J., Costea, D. E., Gammon, L., Fazil, B., Biddle, A., and Mackenzie, I. C., (2010). Normal and malignant epithelial cells with stem-like properties have an extended G2 cell cycle phase that is associated with apoptotic resistance. *BMC Cancer*, vol. 10, article 166.

Haselbeck, R. J., Hoffmann, I., & Duester, G. (1999). Distinct functions for Aldh1 and Raldh2 in the control of ligand production for embryonic retinoid signaling pathways. *Developmental Genetics*, *25*(4), 353–364. http://doi.org/10.1002/(SICI)1520-6408(1999)25:4<353::AID-DVG9>3.0.CO;2-G

Hejmadi, M. (2010). *Introduction to Cancer Biology*. *Bookboon*. http://doi.org/10.1017/CBO9781139012904

Hess, D.A., Meyerrose, T.E., Wirthlin, L., Craft, T.P., Herrbrich, P.E., Creer, M.H., Nolta, J.A., (2004). Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity. *Blood*. 104:pp. 1648–1655.

Hildebrand, L. C., Carvalho, A. L., Lauxen, I. S., Nör, J. E., Cerski, C. T. S., & Sant'ana Filho, M. (2014). Spatial distribution of cancer stem cells in head and neck squamous cell carcinomas. *Journal of Oral Pathology & Medicine : Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, (27), 1–8. http://doi.org/10.1111/jop.12169

Ioachim, E. (1999). Glycoprotein CD44 expression in benign, premalignant and malignant epithelial lesions of the larynx: An immunohistochemical study

including correlation with Rb, p53, Ki-67 and PCNA. *Histology and Histopathology*, *14*(4), 1113–1118.

Karsten, U., & Goletz, S. (2013). What makes cancer stem cell markers different? *Springer Plus*, *2*(1), 301. http://doi.org/10.1186/2193-1801-2-301

Katoh, M., & Katoh, M. (2007). WNT signaling pathway and stem cell signaling network. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *13*(14), 4042–4045. http://doi.org/10.1158/1078-0432.CCR-06-2316

Kaur, J., Rao, M., Chakravarti, N., Mathur, M., Shukla, N. K., Sanwal, B. D., & Ralhan, R. (2001). Co-expression of colligin and collagen in oral submucous fibrosis: Plausible role in pathogenesis. *Oral Oncology*, *37*(3), 282–287. http://doi.org/10.1016/S1368-8375(00)00121-4

Keysar, S. B., & Jimeno, A. (2010). More than markers: biological significance of cancer stem cell-defining molecules. *Molecular Cancer Therapeutics*, *9*(9), 2450–2457. http://doi.org/10.1158/1535-7163.MCT-10-0530

Khan, S., Chatra, L., Prashanth, S. K., Veena, K. M., & Rao, P. K. (2012). Pathogenesis of oral submucous fibrosis. *Journal of Cancer Research and Therapeutics*, *8*(2), 199–203. http://doi.org/10.4103/0973-1482.98970

Klonisch, T., Wiechec, E., Hombach-Klonisch, S., Ande, S. R., Wesselborg, S., Schulze-Osthoff, K., & Los, M. (2008). Cancer stem cell markers in common cancers - therapeutic implications. *Trends in Molecular Medicine*. http://doi.org/10.1016/j.molmed.2008.08.003

Knudson, W., Chow, G., & Knudson, C. B. (2002). CD44-mediated uptake and degradation of hyaluronan. *Matrix Biology*. http://doi.org/10.1016/S0945-053X(01)00186-X

Kreso, A., & Dick, J. E. (2014). Evolution of the cancer stem cell model.*CellStemCell*. http://doi.org/10.1016/j.stem.2014.02.006

Krishnamurthy, S., Dong, Z., Vodopyanov, D., *et al*., (2010). Endothelial cell-initiated signaling promotes the survival and self-renewal of cancer stem cells. *Cancer Res*. 70:pp. 9969–78.

Kung, H.C., Hoyert, D.L., Xu, J.Q., Murphy, S.L., (2008). Deaths: Final data for 2005. *National vital statistics reports*, Vol. 56, No. 10, Hyattsville, MD, National Center for Health Statistics.

Li, C., Heidt, D.G., Dalerba, P., *et al*., (2007). Identification of pancreatic cancer stem cells. *Cancer Res*. 67:pp. 1030–7.

Li, L., & Neaves, W. B. (2006). Normal stem cells and cancer stem cells: the niche matters. *Cancer Research*, *66*(9), 4553–7. http://doi.org/10.1158/0008-5472.CAN-05-3986

Li, Y., Jiang, D., Liang, J., Meltzer, E. B., Gray, A., Miura, R., Noble, P. W. (2011). Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *The Journal of Experimental Medicine*, *208*(7), 1459–1471. http://doi.org/10.1084/jem.20102510

Lingala, S., Cui, Y.-Y., Chen, X., Ruebner, B. H., Qian, X.-F., Zern, M. A., & Wu, J. (2010). Immunohistochemical staining of cancer stem cell markers in hepatocellular carcinoma. *Experimental and Molecular Pathology*, *89*(1), 27–35. http://doi.org/10.1016/j.yexmp.2010.05.005

Lobo, N. a, Shimono, Y., Qian, D., & Clarke, M. F. (2007). The biology of cancer stem cells. *Annual Review of Cell and Developmental Biology*, *23*, 675–99. http://doi.org/10.1146/annurev.cellbio.22.010305.104154

Ma, I., & Allan, A. L. (2011). The role of human aldehyde dehydrogenase in normal and cancer stem cells. *Stem Cell Reviews*, *7*(2), 292–306. http://doi.org/10.1007/s12015-010-9208-4

Macfarlane, G. J., Boyle, P., Evstifeeva, T. V., Robertson, C., & Scully, C. (1994). Rising trends of oral cancer mortality among males worldwide: The return of an

old public health problem. *Cancer Causes and Control*, *5*(3), 259–265. http://doi.org/10.1007/BF01830246

Mahomed, F. (2012). Oral submucous fibrosis--a potentially malignant condition of growing concern. *SADJ : Journal of the South African Dental Association = Tydskrif van Die Suid-Afrikaanse Tandheelkundige Vereniging*, *67*, 562,564–565. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23957096

Mani, N. J., & Singh, B. (1976). Studies on oral submucous fibrosis. III. Epithelial changes. *Oral Surgery, Oral Medicine, and Oral Pathology*, *41*(2), 203–214.

Mannelli, G., and Gallo, O., (2012). Cancer stem cells hypothesis and stem cells in head and neck cancers. *Cancer Treatment Reviews*. 38:pp. 515–539.

Mărgăritescu, C., Pirici, D., Simionescu, C., & Stepan, A. (2012). The utility of CD44, CD117 and CD133 in identification of cancer stem cells (CSC) in oral squamous cell carcinomas (OSCC). *Romanian Journal of Morphology and Embryology*, *52*(3 SUPPL.), 985–993.

Matsui, W., Huff, C.A., Wang, Q., Malehorn, M.T., Barber, J., Tanhehco, Y., Smith, B.D., Civin, C.I., Jones, R.J., (2004). Characterization of clonogenic multiple myeloma cells. *Blood*. 103:pp. 2332–2336.

McAllister, S. S., & Weinberg, R. A. (2010). Tumor-host interactions: A far-reaching relationship. *Journal of Clinical Oncology*. http://doi.org/10.1200/JCO.2010.28.4257

Meacham, C. E., & Morrison, S. J. (2013). Tumour heterogeneity and cancer cell plasticity. *Nature*, *501*(7467), 328–37. http://doi.org/10.1038/nature12624

Medici, D., & Kalluri, R. (2012). Endothelial-mesenchymal transition and its contribution to the emergence of stem cell phenotype. *Seminars in Cancer Biology*. http://doi.org/10.1016/j.semcancer.2012.04.004

Meran, S., Luo, D. D., Simpson, R., Martin, J., Wells, A., Steadman, R., & Phillips, A. O. (2011). Hyaluronan facilitates transforming growth factor-β1-

dependent proliferation via CD44 and epidermal growth factor receptor interaction. *Journal of*

Biological Chemistry, *286*(20), 17618–17630.

http://doi.org/10.1074/jbc.M111.226563

Merchant, A. A., & Matsui, W. (2010). Targeting Hedgehog--a cancer stem cell pathway. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *16*(12), 3130–3140. http://doi.org/10.1158/1078-0432.CCR-09-2846

Minato, T., Yamamoto, Y., Seike, J., Yoshida, T., Yamai, H., Takechi, H., Tangoku, A. (2012). Aldehyde Dehydrogenase 1 Expression is Associated with Poor Prognosis in Patients with Esophageal Squamous Cell Carcinoma. *Annals of Surgical Oncology*. http://doi.org/10.1245/s10434-012-2535-8

Mithani, S. K., Mydlarz, W. K., Grumbine, F. L., Smith, I. M., & Califano, J. A. (2007). Molecular genetics of premalignant oral lesions. *Oral Diseases*. http://doi.org/10.1111/j.1601-0825.2006.01349.x

Mo, J.-S., Park, H. W., & Guan, K.-L. (2014). The Hippo signaling pathway in stem cell biology and cancer. *EMBO Reports*, *15*, 642–56. http://doi.org/10.15252/embr.201438638

Morrison, S. J., & Kimble, J. (2006). Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature*, *441*(7097), 1068–1074. http://doi.org/10.1038/nature04956

Morrison, S. J., & Kimble, J. (2006). Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature*, *441*(7097), 1068–1074. http://doi.org/10.1038/nature04956

Murti, P. R., Bhonsle, R. B., Pindborg, J. J., Daftary, D. K., Gupta, P. C., & Mehta, F. S. (1985). Malignant transformation rate in oral submucous fibrosis over a 17-

year period. *Community Dentistry and Oral Epidemiology*, *13*(6), 340–341. http://doi.org/10.1111/j.1600-0528.1985.tb00468.x

Murti, P.R., Bhonsle, R.B., Pindborg, J.J., Daftary, D.K., Gupta, P.C., *et al*. (1985). Malignant transformation rates in oral submucous fibrosis over a 17 year period. *Community Dent Oral Epidemiol*. 13:pp. 340-1.

Naor, D., Nedvetzki, S., Golan, I., Melnik, L., & Faitelson, Y. (2002). CD44 in cancer. *Critical Reviews in Clinical Laboratory Sciences*, *39*(6), 527–579. http://doi.org/10.1080/10408360290795574

Natarajan, T. G., & FitzGerald, K. T. (2007). Markers in normal and cancer stem cells. *Cancer Biomarkers : Section A of Disease Markers*, *3*(4-5), 211–231.

Oo, H.N., Myint, Y.Y., Maung. C.N., *et al*., (2011). Oral cancer in Myanmar: a preliminary survey based on hospital-based cancer registries. *J Oral Pathol Med*. 40:pp. 20-26.

Orian-Rousseau, V. (2010). CD44, a therapeutic target for metastasising tumours. *European Journal of Cancer*, *46*(7), 1271–1277. http://doi.org/10.1016/j.ejca.2010.02.024

Pardal, R., Clarke, M. F., & Morrison, S. J. (2003). Applying the principles of stem-cell biology to cancer. *Nature Reviews. Cancer*, *3*(12), 895–902. http://doi.org/10.1038/nrc1232

Pindborg, J. J., & Sirsat, S. M. (1966). Oral submucous fibrosis. *Oral Surgery, Oral Medicine, and Oral Pathology*, *22*, 764–79. http://doi.org/10.1016/0030-4220(66)90367-7

Pindborg, J.J., Bhonsle, R.B., Murti, P.R., Gupta, P.C., Daftary, D.K., Mehta, F.S., (1980). Incidence and early forms of oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol*. 50(1):pp. 40–4.

Pindborg, J.J., Sirsat, S.M., (1966): Oral submucous fibrosis. O*ral Surgery Oral Medicine & Oral Pathology*. 22:pp. 764–779.

Polyak, K., & Weinberg, R. A. (2009). Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nature Reviews. Cancer*, *9*(4), 265–273. http://doi.org/10.1038/nrc2620

Ponta, H., Sherman, L., & Herrlich, P. A. (2003). CD44: from adhesion molecules to signalling regulators. *Nature Reviews. Molecular Cell Biology*, *4*(1), 33–45. http://doi.org/10.1038/nrm1004

Prince, M. E., Sivanandan, R., Kaczorowski, A., Wolf, G. T., Kaplan, M. J., Dalerba, P., Ailles, L. E. (2007). Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 973–978. http://doi.org/10.1073/pnas.0610117104

Prince, M.E.P., Ailles, L.E., (2008). Cancer stem cells in head and neck squamous cell cancer. *J Clin Oncol*. 26:pp. 2871–5.

Qi, Y., Li, R. M., Kong, F. M., Li, H., Yu, J. P., & Ren, X. B. (2012). How do tumor stem cells actively escape from host immunosurveillance? *Biochemical and Biophysical Research Communications*. http://doi.org/10.1016/j.bbrc.2012.03.086

Qian, X., Wagner, S., Ma, C., Klussmann, J. P., Hummel, M., Kaufmann, A. M., & Albers, A. E. (2013). ALDH1-positive cancer stem-like cells are enriched in nodal metastases of oropharyngeal squamous cell carcinoma independent of HPV status. *Oncology Reports*, *29*(5), 1777–1784. http://doi.org/10.3892/or.2013.2340

Radisky, D. C., & LaBarge, M. A. (2008). Epithelial-Mesenchymal Transition and the Stem Cell Phenotype. *Cell Stem Cell*. http://doi.org/10.1016/j.stem.2008.05.007

Rajalalitha, P., & Vali, S. (2005). Molecular pathogenesis of oral submucous fibrosis--a collagen metabolic disorder. *Journal of Oral Pathology & Medicine :*

Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology, *34*(6), 321–328. http://doi.org/10.1111/j.1600-0714.2005.00325.x

Rajendran, R. (1994). Oral submucous fibrosis: Etiology, pathogenesis, and future research. *Bulletin of the World Health Organization*.

Reya, T., Morrison, S. J., Clarke, M. F., Weissman, I. L., (2001). Stem cells, cancer, and cancer stem cells. *Nature*, *414*, 105 – 111. http://www.nature.com/nature/journal/v414/n6859/pdf/414105a0.pdf

Saegusa, M., Machida, D., Hashimura, M., & Okayasu, I. (1999). CD44 expression in benign, premalignant, and malignant ovarian neoplasms: Relation to tumour development and progression. *Journal of Pathology*, *189*(3), 326–337. http://doi.org/10.1002/(SICI)1096-9896(199911)189:3<326::AID-PATH425>3.0.CO;2-6

Shackleton, M., Quintana, E., Fearon, E. R., & Morrison, S. J. (2009). Heterogeneity in Cancer: Cancer Stem Cells versus Clonal Evolution. *Cell*. http://doi.org/10.1016/j.cell.2009.08.017

Shah, J.P., Lydiatt, W., (1995). Treatment of cancer of the head and neck. *CA Cancer J Clin.* 45: pp. 352–68.

Shipitsin, M., Campbell, L.L., Argani, P., *et al*., (2007). Molecular definition of breast tumor heterogeneity. *Cancer Cell.* 11:pp. 259–73.

Siegelman, M. H., DeGrendele, H. C., & Estess, P. (1999). Activation and interaction of CD44 and hyaluronan in immunological systems. *Journal of Leukocyte Biology*, *66*(2), 315–321.

Sneddon, J. B., & Werb, Z. (2007). Location, Location, Location: The Cancer Stem Cell Niche. *Cell Stem Cell*. http://doi.org/10.1016/j.stem.2007.11.009

Takahashi-Yanaga, F., & Kahn, M. (2010). Targeting Wnt signaling: Can we safely eradicate cancer stem cells? *Clinical Cancer Research*. http://doi.org/10.1158/1078-0432.CCR-09-2943

Tanaka, T., Tanaka, M., & Tanaka, T. (2011). Oral carcinogenesis and oral cancer chemoprevention: a review. *Pathology Research International*, *2011*, 431246. http://doi.org/10.4061/2011/431246

The National Academies, (2006). *Understanding stem cells*: An overview of the science and issues from the National Academies. Booklet from National Academy of Sciences, National Academy of Engineering, Institute of Medicine, National Research Council.

Themeli, M., Kloss, C. C., Ciriello, G., Fedorov, V. D., Perna, F., Gonen, M., & Sadelain, M. (2013). Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. *Nature Biotechnology*, *31*(10), 928–33. http://doi.org/10.1038/nbt.2678

Tilakaratne, W. M., Klinikowski, M. F., Saku, T., Peters, T. J., & Warnakulasuriya, S. (2006). Oral submucous fibrosis: Review on aetiology and pathogenesis. *Oral Oncology*. http://doi.org/10.1016/j.oraloncology.2005.08.005

Toole, B. P., & Slomiany, M. G. (2008). Hyaluronan, CD44 and Emmprin: Partners in cancer cell chemoresistance. *Drug Resistance Updates*, *11*(3), 110–121. http://doi.org/10.1016/j.drup.2008.04.002

Tsantoulis, P. K., Kastrinakis, N. G., Tourvas, A. D., Laskaris, G., & Gorgoulis, V. G. (2007). Advances in the biology of oral cancer. *Oral Oncology*. http://doi.org/10.1016/j.oraloncology.2006.11.010

Van der Waal I, (2009). Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. *Oral Oncol*. 45:pp. 317-23.

Varkevisser, C. M., Pathmanathan, I., & Brownlee, A. (2003). Module 11: Sampling (IDRC). In DESIGNING AND CONDUCTING HEALTH SYSTEMS RESEARCH PROJECTS: VOLUME 1 Proposal Development and Fieldwork. Canada: International Development Research Center. Retrieved from http://web.idrc.ca/en/ev-33011-201-1-DO_TOPIC.html

Vezzoni, L., & Parmiani, G. (2008). Limitations of the cancer stem cell theory. In *Cytotechnology* (Vol. 58, pp. 3–9). http://doi.org/10.1007/s10616-008-9166-8

Visvader, J. E. (2011). Cells of origin in cancer. *Nature*, *469*(7330), 314–322. http://doi.org/10.1038/nature09781

Visvader, J. E., & Lindeman, G. J. (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nature Reviews. Cancer*, *8*(10), 755–768. http://doi.org/10.1038/nrc2499

Warnakulasuriya, S. (2010). Living with oral cancer: Epidemiology with particular reference to prevalence and life-style changes that influence survival. *Oral Oncology*. http://doi.org/10.1016/j.oraloncology.2010.02.015

Warnakulasuriya, S., (2001). Histological grading of oral epithelial dysplasia: revisited. *Journal of Pathology.*194:pp. 294–297.

Williams, K., Motiani, K., Giridhar, P. V., & Kasper, S. (2013). CD44 integrates signaling in normal stem cell, cancer stem cell and (pre)metastatic niches. *Experimental Biology and Medicine (Maywood, N.J.)*, *238*(3), 324–38. http://doi.org/10.1177/1535370213480714

Woodward, W. A., & Sulman, E. P. (2008). Cancer stem cells: Markers or biomarkers? *Cancer and Metastasis Reviews*. http://doi.org/10.1007/s10555-008-9130-2

World Health Organization, (2008). Cancer Fact sheet. World Health Organization. http://www.who.int/mediacentre/factsheets/fs297/en/. [Accessed January 19, 2009].

Xu, Z., Shen, Z., Shi, L., Sun, H., Liu, W., Zhou, Z., (2013). ALDH1 expression correlated with malignant potential of oral lichen planus. *Annals of Diagnostic Pathology*. 17;pp. 408-411.

Yao, T., Chen, Q., Zhang, B., Zhou, H., & Lin, Z. (2011). The expression of ALDH1 in cervical carcinoma. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research*, *17*(8), HY21–Y26. http://doi.org/881886 [pii]

Yoo, M.-H., & Hatfield, D. L. (2008). The cancer stem cell theory: is it correct? *Molecules and Cells*, *26*(5), 514–516. http://doi.org/10.1038/leu.2008.198.HOX

Yoshida, A., (1992). Molecular genetics of human aldehyde dehydrogenase. *Pharmacogenetics.* 2:pp. 139–147.

Yoshida, A., Hsu, L. C., & Davé, V. (1992). Retinal oxidation activity and biological role of human cytosolic aldehyde dehydrogenase. *Enzyme*, *46*(4-5), 239–244.

APPENDICES

1. CONSENT FORM

Informed Consent from (In English)

Part (A) Statement

(1) Purpose

Oral submucous fibrosis and oral squamous cell carcinoma are the most common in oral cavity. The purpose of this study is to better information about of oral submucous fibrosis (OSMF) and oral squamous cell carcinoma (OSCC) so that more effective treatment can be given in the future.

(2) Participant selection

All patients whose biopsies sent to Department of Oral Medicine, U.D.M (Yangon) diagnosed as OSMF and OSCC.

(3) Procedures

The biopsy specimens and paraffin sections sent to the Department of Oral Medicine U.D.M (Yangon) will be studied after the official issue of the histological diagnosis. There will be no interference with treatment of the patient.

(4) Risks

By participating in this study, there will be no risk nor suffering for patients because the study will be only done on the biopsy specimens which is left after the official issue of the histological diagnosis.

(5) Discomforts

There will be no additional discomfort.

(6) Benefits

Results of the study may aid in prognosis of the patient with OSMF and OSCC may support better treatment.

(7) Incentives

By participating in this study, any money nor special facilities will not be given and all the procedures will be done free of charge for the patients.

(8) Compensation

Compensation of any sort will not be provided for participating in this study.

(9) Confidentiality

The information that we collect from this research will be kept confidential. Information about you will be collected during the research will be put away and no-one but the researcher will be able to see it. Any information about you will have a number on it instead of your name.

A copy of this informed consent form has been provided to the patient.

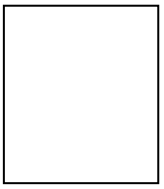
Part (B) Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and questions that I have asked have been answered to my satisfaction. I have been informed that there is no risk. I am aware that there may be no benefit to me personally. I have been provided the phone number and address of the researcher for easy contact.

The result of the research will not be relayed back to the patient and will be used only for the research paper presentation and publication in order interested people may learn from the research. I give my consent voluntarily to participate as a participant in this research and understand that I have the right to withdraw from the study at any time without in any way affecting my medical care.

Signature of Patient -

Thumb print of participant -



Name of Patient -

Date (day, month, year) -

I confirmed that the above patient has been properly informed and voluntarily consented to participate in this study.

Signature of witness -

Name of witness -

Date (day, month, year) -

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Signature of Researcher -

Name of Researcher - Dr. Khin Soe
B.D.S, M.D.Sc
U.D.M (Mandalay)

Date (day, month, year) -

Informed consent form (in Myanmar)

သဘောတူညီချက်ပုံစံ

အပိုင်း (က) သုတေသနအချက်အလက်များ

(၁) သုတေသနအမည်

ခံတွင်းအကြိုကင်ဆာ (OSMF) နှင့်ခံတွင်းကင်ဆာရောဂါဖြစ်စဉ်နှင့် Cancer Stem Cell ဆက်နွယ်မှုအားလေ့လာသောသုတေသန။

(၂) သုတေသနလုပ်ငန်းရည်ရွယ်ချက်သုတေသနလုပ်ငန်းရည်ရွယ်ချက်မှာခံတွင်းကင်ဆာရောဂါနှင့် (OSMF) အကြောင်းရင်းကို ပိုမိုသိရှိလာစေပြီးနောင်တွင် ၎င်းရောဂါများကို ထိရောက်သောကုထုံးများ ဖြင့်ကုသ နိုင်ရန်ဖြစ်ပါသည်။

(၃) သုတေသနတွင်ပါဝင်မည့်သူများကို ရွေးချယ်ခြင်း။

သွားဘက်ဆိုင်ရာဆေးတက္ကသိုလ်၊ ရန်ကုန်မေးရိုးနှင့် ခံတွင်းဆေးပညာဌာနသို့ ပို့ဆောင်ဖြေရှာသည့် အသားစနမူနာများအနက် ခံတွင်းအကြိုကင်ဆာ(OSMF) နှင့်ခံတွင်းကင်ဆာရောဂါဟု ရောဂါအမည်တပ် ခြင်းခံရသည့် လူနာများအားလုံးကို ဤသုတေသနတွင် ပါဝင်ရန် ဖိတ်ခေါ်အပ်ပါသည်။

(၄) သုတေသနလုပ်ငန်း ဆောင်ရွက်ရန်။

သွားဘက်ဆိုင်ရာဆေးတက္ကသိုလ်၊ ရန်ကုန်မေးရိုးနှင့် ခံတွင်းဆေးပညာ ဌာနသို့ပို့ဆောင်ဖြေရှာသည့်ခံတွင်းအကြိုကင်ဆာ(OSMF)နှင့် ခံတွင်းကင်ဆာအသားစနမူနာများနှင့် တရားဝင်ရောဂါ အမည်တပ်၍ အဖြေထုတ်ပေးပြီးနောက်ကြွင်းကျန်သောဖယောင်းတွင်းမှအသားစများကိုများကိုသာ သုတေသန ပြုလုပ်မည်ဖြစ် ပါသည်။ဤသုတေတန လုပ်ငန်းကြောင့်လူနာအားကုသမှု နောင့်နှေးခြင်းမရှိပါ။

(၅) **ထေးထွက်ဆိုးကျိုး**

သွားဘက်ဆိုင်ရာဆေးတက္ကသိုလ်၊ ရန်ကုန်မေးရိုးနှင့်ခံတွင်းဆေးပညာဌာနမှ တရားဝင်ရောဂါ အမည် တပ်၍ အဖြေထုတ်ပေးပြီးမှ ကြွင်းကျန်သောဖယောင်းတွင်းမှ အသားစများကိုသာ သုတေသနပြုလုပ်မည်ဖြစ်ရာ မလိုလား အပ်သော ဘေးထွက်ဆိုးကျိုး တစ်စုံတစ်ရာမရှိပါ။

(၆) ဖြစ်ပေါ်နိုင်ထော အန္တရာယ်ရှိမှု

ဤသုတေသနဌာနတွင်ပါဝင်ခြင်းဖြင့်လူနာတွင်မည်သည့်အန္တရာယ်ရာယ်မျှ မရှိပါ။

(၇) အကျိုးကျေးဇူး

သုတေသနမှ တွေ့ရှိချက်များသည် ခံတွင်းကင်ဆာရောဂါအကြောင်းကို ပိုမိုသိရှိလာစေပြီး နောင်တွင် ၎င်းရောဂါကိုပိုမိုထိရောက်သောကုထုံးများဖြင့်ကုသနိုင်မည်ဖြစ်ပါသည်။

(၈) လူနာများအတွက်လက်ငင်းရရှိနိုင်မည့် အကျိုးအမြတ်

ဤသုတေသနလုပ်ငန်းတွင် ပါဝင်ခြင်းဖြင့် အဆင့်မြင့်ဓါတ်ခွဲစမ်းသပ်ချက်များကို ငွေကြေးတစ်စုံတစ်ရာ ကုန်ကျခြင်းမရှိပဲ ဆောင်ရွက်ပေးမည်ဖြစ်ပါသည်။

(၉) ကျော်ကြေးခံစားခွင့်

ဤသုတေသနဌာနတွင် ပါဝင်ခြင်းကြောင့် ငွေကြေး (သို့မဟုတ်) အထူးအခွင့်အရေး ပေးမည်မဟုတ်ပါ။ မည်သည့်ကျော်ကြေး ခံစားခွင့်ကိုမျှလည်း ပေးမည် မဟုတ်ပါ။

(၁၀) လျှို့ဝှက်ထားရှိမှု

သုတေသနစီမံချက်မှရရှိသော သတင်းအချက်အလတ်များကို လျှို့ဝှက်ထားပါမည်။ အချက်အလက်များ ကို သုတေသနပြုလုပ်သူများမှလွဲ၍ မည်သူတစ်ဦးတစ်ယောက်ကမျှ ကြည့်ပိုင်ခွင့်မရှိပါ။ အမည်အစား နံပါတ်ဖြင့်အချက်အလတ်များကိုသိမ်းဆည်း ထားပါမည်။

အပိုင်း (ခ) သဘောတူညီချက်ပုံစံ

ကျွန်ုပ်သည် ရေမှုအချက်အလက်များကို ဖတ်ရှုပြီးဖြစ်ပြီး (သို့မဟုတ်) ဖတ်ပြပြီးဖြစ်ပြီး ကျွန်ုပ်တွင်မေးခွန်း မေးပိုင်ခွင့်နှင့် ထိုမေးခွန်းများကို ကျွန်ုပ်ကျေနပ်သည်အထိ ဖြေကြားပေးမည်ကိုသိရှိပြီး ဖြစ်ပါသည်။ ယင်းသုတေသနတွင် ပါဝင်ခြင်းဖြင့် မည်သည့်အန္တရာယ်မျှမရှိကြောင်း၊ မည်သည့်ပုဂ္ဂိုလ်ရေးဆိုင်ရာအကျိုး ကျေးဇူး ရရှိမည်မဟုတ်ကြောင်းနှင့် မည်သည့်ကျော်ကြေးမျှလည်းခံစားခွင့်မရှိ ကြောင်းသိရှိပြီးဖြစ်သည်။ ကျွန်ုပ်အား သုတေသနပြုလုပ်သူနှင့် ဆက်သွယ်ရန်လိပ်စာနှင့် ဖုန်းနံပါတ်များကိုပေးထားပါသည်။ ကျွန်ုပ်၏ရောဂါနှင့် ပတ်သက်၍ လေ့လာတွေ့ရှိချက်များအား သုတေသနစာတမ်းတွင် ထည့်သွင်းအသုံးပြုခြင်းမှလွဲ၍သုတေသီမှ လျှို့ဝှက်ထားမည်ဟု သိရှိရပါသည်။ ကျွန်ုပ်သည်ဤသုတေသနတွင် မိမိသဘောအလျောက်ပါဝင်ရန် သဘောတူပါသည်။ ဤသုတေသနလုပ်ငန်းမှ အချိန်မရွေး နုတ်ထွက်ခွင့်ရှိကြောင်း နားလည်ပြီးဖြစ်၍ ဤသုတေ သနတွင်ပါဝင်ရန် သဘောတူလက်မှတ်ရေးထိုးပါသည်။

လူနာ၏လက်မှတ် –

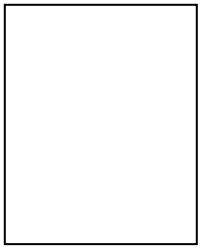
လူနာ၏အမည် –

နိုင်ငံသားမှတ်ပုံတင်အမှတ် –

အကယ်၍လူနာသည်စာမတတ်သူဖြစ်လျှင် –

သုတေသနတွင်ပါဝင်မည့်သူ၏လက်ဗွေ –

နေ့စွဲ –



အထက်ပါလူနာသည် ဤသုတေသနနှင့် ပတ်သက်သည့်အကြောင်းအရာများကို သေချာစွာ နားလည်သိရှိပြီး ၎င်း၏သဘောအလျောက် ဤသုတေသနတွင်ပါဝင်ရန် သဘောတူညီကြောင်း ကျွန်ုပ်က သက်သေပြပါသည်။

သက်သေ၏လက်မှတ် –

သက်သေ၏အမည် –

နေ့စွဲ –

သုတေသီ၏လက်မှတ် –

သုတေသီ၏အမည်/ရာထူး

2. List of OSMF cases and staining results

No	Code	Reg. No	Diagnosis	CD44				ALDH1				Co-expression
				1	2	3	4	1	2	3	4	
1	001	266/14	OSMF				4	1				
2	002	135/14	OSMF				4	1				
3	003	49/14	OSMF				4	1				
4	004	105/14	OSMF				4		2			*
5	005	188/14	OSMF				4	1				
6	006	196/14	OSMF			4			2			*
7	007	134/14	OSMF				4		2			*
8	008	197/14	OSMF				4	1				
9	009	155/14	OSMF				4		2			*
10	010	156/14	OSMF				4			3		*
11	011	169/14	OSMF		2					4		*
12	012	211/14	OSMF			3			2			*
13	013	212/14	OSMF				4	1				
14	014	251/14	OSMF				4		2			*
15	015	252/14	OSMF			3				4		*
16	016	253/14	OSMF				4		2			*
17	017	264/14	OSMF		2			1				
18	018	293/14	OSMF				4			4		*
19	019	132/14	OSMF				4		2			*
20	020	334/13	OSMF			4				4		*
21	021	103/13	OSMF	1				1				
22	022	227/11	OSMF				4		2			*
23	023	197/11	OSMF				4		2			*
24	024	144/11	OSMF				4		2			*
25	025	137/11	OSMF				4		2			*
26	026	104/13	OSMF	1				1				
27	027	198/14	OSMF	1				1				
28	028	213/14	OSMF	1				1				
29	029	204/14	OSMF	1				1				
30	030	30/14	OSMF	1				1				
				20%	6.67 %	13.33%	60%	43.33%	40%	16.67%		56.67 % (n=17)

3.List of OSCC cases and staining results

No	Code	Reg. No	Diagnosis	CD44				ALDH1				Co-staining	
				1	2	3	4	1	2	3	4		
1	031	171/14	OSCC				4			3		*	
2	032	180/14	OSCC				4			2		*	
3	033	187/14	OSCC				4		2			*	
4	034	165/14	OSCC				4	1					
5	035	98/14	OSCC				4			2		*	
6	036	70/14	OSCC				4		3			*	
7	037	116/14	OSCC				4		3			*	
8	038	10/14	OSCC				4		2			*	
9	039	443/13	OSCC	1						3			
10	040	411/13	OSCC	1				1					
11	041	416/13	OSCC	1				1					
12	042	312/13	OSCC	1					2				
13	043	328/13	OSCC	1					2				
14	044	341/13	OSCC		2				2			*	
15	045	446/14	OSCC	1					2				
16	046	278/14	OSCC	1				1					
17	047	328I/14	OSCC		2					3		*	
18	048	320/13	OSCC		3				3			*	
19	049	300/13	OSCC	1				1					
20	050	445/14	OSCC	1				1					
21	051	464/13	OSCC	1				1					
22	053	334/13	OSCC			3				3		*	
23	054	302/13	OSCC	1				1					
24	055	301//13	OSCC	1					2				
25	056	320/13	OSCC		2					2		*	
26	057	418/13	OSCC	1				1					
27	058	356/13	OSCC	1				1					
28	059	311/13I	OSCC		2			1					
29	060	311/13II	OSCC		3			1					
30	061	296/13	OSCC		1			1					
31	062	289/13	OSCC		1			1					
32	063	278/13	OSCC		1				1			*	
33	064	314/13	OSCC		2				2			*	
34	065	269	OSCC		2			1					

4. List of control cases and staining results

No	Code	Diagnosis	CD44				ALDH1				Co-staining	Remark
			1	2	3	4	1	2	3	4		
1	269/13	Leukoplakia	1				1					
2	361/14	Pyogenic granuloma		2			1					
3	414/13	Reactive fibroepithelial hyperplasia	1				1					
4	375/13	Mucocele	1				1					
5	261/14	Mucocele	1				1					
6	192/14	Pyogenic granuloma	1					2				
7	243/14	Mucocele	1				1					
8	247/14	Mucocele	1				1					
9	261/14	Mucocele	1				1					
10	263/14	Reactive fibroepithelial hyperplasia	1				1					
11	268/14	Mucocele	1				1					
12	10/13	Nonspecific	1				1					
13	24/13	Mucocele	1				1					
14	25/13	Fibroepithelial hyperplasia	1				1					
15	28/13	Mucocele	1				1					

5. Spearman’s rho correlation in OSMF

Correlations

			Age	Sex	Duration	Lymphocytes	Hyalinization	Compression	Fibroblasts	Vessels	Atrophy	CD44	ALDH1	Costaining
Spearman's rho	Age	Correlation Coefficient	1.000	.379*	.088	-.205	-.226	-.238	-.004	.405*	.349	-.069	-.264	-.257
		Sig. (2-tailed)	.	.039	.644	.277	.229	.206	.982	.027	.059	.718	.159	.170
		N	30	30	30	30	30	30	30	30	30	30	30	30
	Sex	Correlation Coefficient	.379*	1.000	-.303	.032	.102	-.255	-.154	.176	.446*	.121	-.217	-.154
		Sig. (2-tailed)	.039	.	.104	.866	.590	.174	.415	.352	.014	.523	.250	.417
		N	30	30	30	30	30	30	30	30	30	30	30	30
	Duration	Correlation Coefficient	.088	-.303	1.000	.208	.102	-.056	.053	-.042	.133	-.058	.270	.174
		Sig. (2-tailed)	.644	.104	.	.269	.592	.770	.780	.825	.483	.762	.149	.357
		N	30	30	30	30	30	30	30	30	30	30	30	30
	Lymphocytes	Correlation Coefficient	-.205	.032	.208	1.000	.152	-.193	-.031	-.183	-.144	-.166	.000	-.110

	Sig. (2-tailed)	.277	.866	.269	.	.424	.307	.872	.334	.447	.381	1.000	.563
	N	30	30	30	30	30	30	30	30	30	30	30	30
Hyalinization	Correlation Coefficient	-.226	.102	.102	.152	1.000	-.102	-.308	-.415*	.131	-.155	.197	.212
	Sig. (2-tailed)	.229	.590	.592	.424	.	.590	.098	.023	.489	.414	.296	.260
	N	30	30	30	30	30	30	30	30	30	30	30	30
Compression	Correlation Coefficient	-.238	-.255	-.056	-.193	-.102	1.000	-.202	.035	-.279	-.212	-.182	-.164
	Sig. (2-tailed)	.206	.174	.770	.307	.590	.	.284	.853	.136	.260	.335	.385
	N	30	30	30	30	30	30	30	30	30	30	30	30
Fibroblasts	Correlation Coefficient	-.004	-.154	.053	-.031	-.308	-.202	1.000	-.067	.213	.213	.297	.223
	Sig. (2-tailed)	.982	.415	.780	.872	.098	.284	.	.723	.258	.259	.111	.236
	N	30	30	30	30	30	30	30	30	30	30	30	30
Vessels	Correlation Coefficient	.405*	.176	-.042	-.183	-.415*	.035	-.067	1.000	.253	-.063	-.095	-.150

	Sig. (2-tailed)	.027	.352	.825	.334	.023	.853	.723	.	.177	.740	.617	.428
	N	30	30	30	30	30	30	30	30	30	30	30	30
Atrophy	Correlation Coefficient	.349	.446*	.133	-.144	.131	-.279	.213	.253	1.000	.227	.239	.190
	Sig. (2-tailed)	.059	.014	.483	.447	.489	.136	.258	.177	.	.228	.204	.314
	N	30	30	30	30	30	30	30	30	30	30	30	30
CD44	Correlation Coefficient	-.069	.121	-.058	-.166	-.155	-.212	.213	-.063	.227	1.000	.350	.475**
	Sig. (2-tailed)	.718	.523	.762	.381	.414	.260	.259	.740	.228	.	.058	.008
	N	30	30	30	30	30	30	30	30	30	30	30	30
ALDH1	Correlation Coefficient	-.264	-.217	.270	.000	.197	-.182	.297	-.095	.239	.350	1.000	.930**
	Sig. (2-tailed)	.159	.250	.149	1.000	.296	.335	.111	.617	.204	.058	.	.000
	N	30	30	30	30	30	30	30	30	30	30	30	30
Costaining	Correlation Coefficient	-.257	-.154	.174	-.110	.212	-.164	.223	-.150	.190	.475**	.930**	1.000

	Sig. (2-tailed)	.170	.417	.357	.563	.260	.385	.236	.428	.314	.008	.000	.
	N	30	30	30	30	30	30	30	30	30	30	30	30

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

6. Spearman’s rho correlation in OSCC

Correlations

			Keratinization	NP	Mitosis	Border	Lymphoplasmacyte	Total	CD44	ALDH1	Coexpression	Age	Sex	Duration	Bryne
Spearman's rho	Keratinization	Correlation Coefficient	1.000	.630**	-.069	.595**	.133	.735**	-.116	-.063	-.052	.126	-.118	-.266	.814**
		Sig. (2-tailed)	.	.000	.699	.000	.454	.000	.515	.723	.769	.477	.506	.128	.000
		N	34	34	34	34	34	34	34	34	34	34	34	34	34
	NP	Correlation Coefficient	.630**	1.000	.113	.754**	.114	.812**	-.113	-.097	.083	.096	-.159	-.131	.789**
		Sig. (2-tailed)	.000	.	.526	.000	.520	.000	.524	.587	.640	.588	.369	.459	.000
		N	34	34	34	34	34	34	34	34	34	34	34	34	34
	Mitosis	Correlation Coefficient	-.069	.113	1.000	.082	.033	.312	.231	.349*	.448**	.033	.208	.109	.032

	Sig. (2-tailed)	.699	.526	.	.645	.855	.072	.189	.043	.008	.853	.239	.540	.855
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Border	Correlation Coefficient	.595**	.754**	.082	1.000	-.085	.809**	-.132	-.220	-.052	.067	.000	-.057	.819**
	Sig. (2-tailed)	.000	.000	.645	.	.635	.000	.457	.212	.771	.704	1.000	.750	.000
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Lymphoplasmacyte	Correlation Coefficient	.133	.114	.033	-.085	1.000	.341*	.243	.459**	.382*	-.062	.103	-.057	.358*
	Sig. (2-tailed)	.454	.520	.855	.635	.	.048	.165	.006	.026	.726	.561	.751	.037
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Total	Correlation Coefficient	.735**	.812**	.312	.809**	.341*	1.000	.018	.049	.182	.055	.026	-.117	.945**

	Sig. (2-tailed)	.000	.000	.072	.000	.048	.	.919	.782	.304	.758	.885	.511	.000
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
CD44	Correlation Coefficient	-.116	-.113	.231	-.132	.243	.018	1.000	.373*	.635**	.243	.258	.233	-.074
	Sig. (2-tailed)	.515	.524	.189	.457	.165	.919	.	.030	.000	.166	.140	.184	.678
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
ALDH1	Correlation Coefficient	-.063	-.097	.349*	-.220	.459**	.049	.373*	1.000	.747**	.030	.081	.056	-.003
	Sig. (2-tailed)	.723	.587	.043	.212	.006	.782	.030	.	.000	.868	.651	.752	.984
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Coexpression	Correlation Coefficient	-.052	.083	.448**	-.052	.382*	.182	.635**	.747**	1.000	.217	.328	.208	.037

	Sig. (2-tailed)	.769	.640	.008	.771	.026	.304	.000	.000	.	.218	.059	.238	.834
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Age	Correlation Coefficient	.126	.096	.033	.067	-.062	.055	.243	.030	.217	1.000	.301	.240	.035
	Sig. (2-tailed)	.477	.588	.853	.704	.726	.758	.166	.868	.218	.	.084	.172	.843
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Sex	Correlation Coefficient	-.118	-.159	.208	.000	.103	.026	.258	.081	.328	.301	1.000	.419*	-.039
	Sig. (2-tailed)	.506	.369	.239	1.000	.561	.885	.140	.651	.059	.084	.	.014	.827
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Duration	Correlation Coefficient	-.266	-.131	.109	-.057	-.057	-.117	.233	.056	.208	.240	.419*	1.000	-.172

	Sig. (2-tailed)	.128	.459	.540	.750	.751	.511	.184	.752	.238	.172	.014	.	.330
	N	34	34	34	34	34	34	34	34	34	34	34	34	34
Bryne	Correlation Coefficient	.814**	.789**	.032	.819**	.358*	.945**	-.074	-.003	.037	.035	-.039	-.172	1.000
	Sig. (2-tailed)	.000	.000	.855	.000	.037	.000	.678	.984	.834	.843	.827	.330	.
	N	34	34	34	34	34	34	34	34	34	34	34	34	34

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

7. Kruskal-Wallis Test for CD44 and ALDH1 in OSMF

Ranks

	ALDH1	N	Mean Rank
CD44	1	13	11.27
	4	12	20.25
	9	1	22.00
	12	4	13.38
	Total	30	

Test Statistics[a,b]

	CD44
Chi-Square	8.980
df	3
Asymp. Sig.	.030

a. Kruskal Wallis Test

b. Grouping Variable: ALDH1

Ranks

	CD44	N	Mean Rank
ALDH1	1	6	7.00
	2	1	7.00
	4	2	17.75
	9	2	24.00
	12	2	24.00
	16	17	16.74
	Total	30	

Test Statistics[a,b]

	ALDH1
Chi-Square	12.565
df	5
Asymp.	.028

8. Kruskal Wallis Test for CD44 and ALDH1 in OSCC

Ranks

	ALDH1	N	Mean Rank
CD44	1	15	14.23
	2	1	15.00
	4	8	16.00
	6	5	27.80
	9	5	19.90
	Total	34	

Test Statistics[a,b]

	CD44
Chi-Square	8.304
df	4
Asymp. Sig.	.081

a. Kruskal Wallis Test

b. Grouping Variable: ALDH1

Ranks

	CD44	N	Mean Rank
ALDH1	1	14	13.29
	2	1	16.00
	4	7	21.14
	6	3	16.00
	16	9	21.89
	Total	34	

Test Statistics[a,b]

	ALDH1
Chi-Square	5.900
df	4
Asymp. Sig.	.207

a. Kruskal Wallis Test
b. Grouping Variable: CD44

9. Chi-square test for OSMF

Test Statistics

	Lymphocytes	Hyalinization	Compression	Fibroblasts	Vessels	Atrophy	CD44	ALDH1	Costaining	Age	Sex	Duration
Chi-Square	1.200[a]	26.133[a]	8.533[a]	6.533[a]	13.333[a]	3.333[a]	37.600[b]	14.000[c]	.533[a]	13.067[d]	8.533[a]	26.600[e]
df	1	1	1	1	1	1	5	3	1	16	1	2
Asymp. Sig.	.273	.000	.003	.011	.000	.068	.000	.003	.465	.668	.003	.000

Test Statistics

	Lymphocytes	Hyalinization	Compression	Fibroblasts	Vessels	Atrophy	CD44	ALDH1	Costaining	Age	Sex	Duration
Chi-Square	1.200[a]	26.133[a]	8.533[a]	6.533[a]	13.333[a]	3.333[a]	37.600[b]	14.000[c]	.533[a]	13.067[d]	8.533[a]	26.600[e]
df	1	1	1	1	1	1	5	3	1	16	1	2
Asymp. Sig.	.273	.000	.003	.011	.000	.068	.000	.003	.465	.668	.003	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 15.0.

b. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 5.0.

c. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 7.5.

d. 17 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.8.

e. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 10.0.

10. Chi-square test for OSCC

Test Statistics

	Keratinization	NP	Mitosis	Border	Lymphoplasmacyte	Total	CD44	ALDH1	Coexpression	Age	Sex	Duration	Bryne
Chi-Square	13.294[a]	28.588[a]	9.588[b]	10.706[a]	10.471[a]	9.941[c]	15.412[d]	16.000[d]	1.059[e]	10.000[f]	16.941[e]	30.118[e]	22.118[c]
df	3	3	2	3	3	8	4	4	1	21	1	1	8
Asymp. Sig.	.004	.000	.008	.013	.015	.269	.004	.003	.303	.979	.000	.000	.005

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 8.5.

b. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 11.3.

c. 9 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 3.8.

d. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 6.8.

e. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 17.0.

f. 22 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.5.