Targeting Myeloid Cells for Potential Cancer Therapies

Jingwen Pan[1,*], Yida Zhang[2,#], Wenkai He[3,#] and Yian Chen[4]
[1]*Department of Bioengineering University of California San Diego, CA, U.S.A.*
[2]*Truro High School Truro, Cornwall, U.K.*
[3]*Shanghai Southwest Weiyu Middle School, Shanghai, China*
[4]*IB program Beijing National Day School, Beijing, China*

[#]*These two authors contributed equally to this work and should be considered as co-second authors*

Keywords: Cancer, Myeloid Cells, CXCL12, Oral Squamous Cell Carcinoma, Positron Emission Tomography.

Abstract: Cancer is one of the major health concerns facing the global society. Though significant improvement in cancer therapies have been done, further investigation into alternative treatment approaches are expected to improve clinical outcomes. Myeloid cells are key components that shape the tumor microenvironment. The distribution and recruitment of myeloid cells account for tumor progression and metastasis. Previous studies provided evidence suggesting that the CXCL12/CXCR4 pathway is responsible for recruiting tumor-supportive M2-type macrophages in the oral squamous cell carcinoma (OSCC) model, promoting proliferation and spreading of OSCC cancer cells. CXCL12/CXCR4 pathway also actively affect other types of tumors, like breast cancer. Thus, modifying CXCL12/CXCR4 interaction might be a potential target for cancer treatment. Due to the lack of existing therapies targeting this pathway, we propose a potential treatment targeting the CXCL12/CXCR4 pathway that binds monoclonal antibodies to CXCL12 ligands to eliminate its expression in tumor sites. Positron emission tomography (PET) imaging enables the monitoring and verification of the outcome of this novel design.

1 INTRODUCTION

Cancer, a collection of diseases characterized by an abnormal cell that divides and spread into surrounding tissues uncontrollably, is one of the primary causes of death worldwide (World Health Organization, 2021). Prevailing types of cancer include oral squamous cell carcinoma (OSCC), breast cancer, and melanoma. OSCC is the most common cancer type that contributes to 95% of head and neck cancers largely due to alcohol abuse and smoking (Taghavi and Yazdi, 2015). Though advanced treatments have been developed, the percentage of patients who are alive five years after starting treatment is still unsatisfactory due to the high recurrence and metastasis rate (Taghavi and Yazdi, 2015; National Cancer Institute). Breast cancer is one of the most often diagnoses among female cancer patients and the leading cause of cancer mortality in women (Akram, Iqbal, Daniyal, Khan, 2017). In 2012, around 1.7 million new cases were diagnosed globally, accounting for 25% of all female cancer cases (Breast cancer | world cancer research fund international, 2021). Melanoma is a form of skin cancer with a high incidence of metastasis that could significantly reduce the survival rate (Davis, Shalin, Tackett, 2019). Further understanding and development of cancer mechanisms and treatments are needed to boost patients' chances for survival and recovery.

Myeloid cells are a vital component in the tumor microenvironment (TME). They are derived from a common myeloid progenitor in the human bone marrow (Figure 1) (A., T. (n.d.), 2021). Tumor growth is enhanced by myeloid cells since they promote tumor angiogenesis, accelerate cancer cell migration, and weaken the immune system (Schmid, Varner, 2010).

Pan, J., Zhang, Y., He, W. and Chen, Y.
Targeting Myeloid Cells for Potential Cancer Therapies.
DOI: 10.5220/0011239000003438
In *Proceedings of the 1st International Conference on Health Big Data and Intelligent Healthcare (ICHIH 2022)*, pages 173-180
ISBN: 978-989-758-596-8

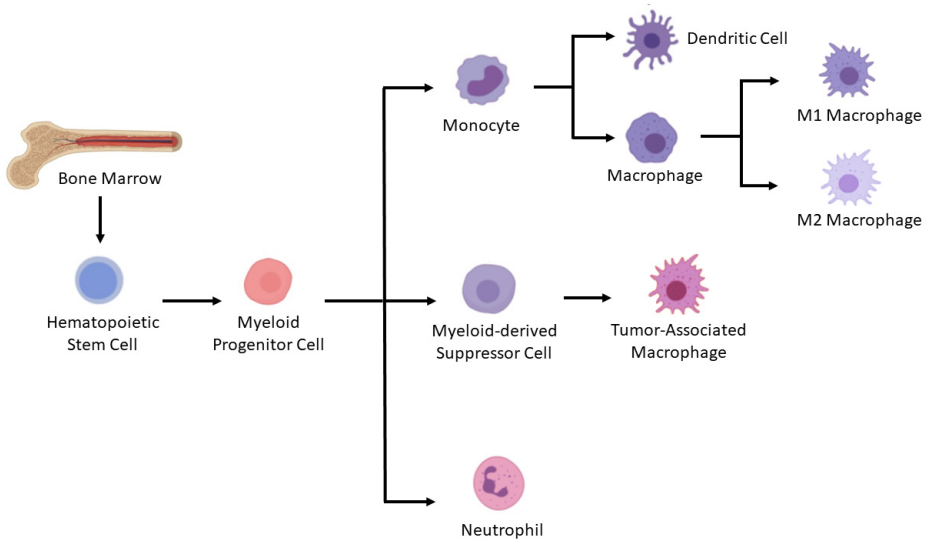

Figure 1. Differentiation of myeloid lineage cells. In the bone marrow, myeloid progenitor cells are derived from hematopoietic stem cells. And then myeloid progenitor cells differentiate into monocyte and neutrophils. Under pathological conditions like cancer, myeloid-derived suppressor cells, which could further differentiate into tumor-associated macrophages, would be generated as well. Macrophages could be induced to derive into either M1 or M2 macrophages. Figure modified from (A., T. (n.d.). 2021).

Myeloid lineage cells comprise a heterogeneous group of cells, including but not limited to macrophages, neutrophils, and myeloid-derived suppressor cells (MDSC). Tumor-associated macrophages (TAMs) are the most copious tumor-infiltrating myeloid cells that work in the innate immune system and assist the initiation of adaptive immunity (A., T. (n.d.). 2021). Based on their functions in tumors and activations, macrophages are classified into two main subsets: classically-activated (M1) macrophages that show tumor-suppressive functions and alternatively activated (M2) macrophages that suppress the immune response and facilitate tumor growth and invasion (Schmid, Varner 2010, Dandekar, Kingaonkar, Dhabekar 2011) . Since TAMs have been proved to contribute to immunosuppression and tumor invasion, the mechanism of how TAMs are recruited to the tumor sites is crucial to the development of potential cancer immunotherapies (Dandekar, Kingaonkar, Dhabekar 2011).

Myeloid-derived suppressor cells (MDSC) constitute a more recently discovered immature myeloid cell population featured by the ability to inhibit immune responses (Lv, Wang, Huang, 2019). MDSC are widely considered as pro-tumorigenic in solid tumors (Lv, Wang, Huang, 2019). These cells demonstrate the pathological state of activation of monocytes and relatively immature neutrophils. Their prominent characteristic is the ability to inhibit T cells' normal functioning, and consequently promote the pathogenesis of various diseases (Veglia, Perego, Gabrilovich, 2018).

Neutrophils are a type of granulocyte and belong to the category of leukocytes. Granulocytes can be classified by their performance under Wright's stain, which is a hematologic stain that used for distinguishing among blood cell types, into three categories: neutrophils, eosinophils, and basophils, of which neutrophils are the most common phagocytic cells (Morris 2018, Wright stain 2020). The bone marrow does not produce neutrophils directly at the onset of infection, but first produces myeloid and promyelocytes, which later differentiate into neutrophils. After receiving the corresponding signal, neutrophils travel through the bloodstream to the infection and proceed to rapidly surround and engulf the foreign material covered by complement and antibodies (Brostjan, Oehler 2020).

Cancer-associated fibroblasts (CAFs), a type of stroma cells that remodel the extracellular matrix, are abundant in the tumor mesenchyme (Liu et al 2019, Ping et al 2021). Accumulating studies indicate that CAFs hinder the function of anti-tumor immunity via their interaction with natural killer (NK) cells and T cells (Li et al 2019). Furthermore, CAFs are responsible for directing immunosuppressive cells onto the tumor owing to their ability to secrete various growth factors and proinflammatory cytokines, such as CXCL12 (Liu et al 2019).

CAF-derived CXCL12 is a type of small molecule chemokines that are distributed in assorted tissues. Similar to other chemokines, CXCL12 along with its chemokine receptor CXCR4 are key factors that mediate the metastasis and proliferation of cancer cells (Righetti 2019, Mollica Poeta, V., Massara, M., Capucetti, A., & Bonecchi, R. 2019). Recent studies revealed that the activation of the CXCL12/CXCR4 pathway positively correlates with the recruitment of TAMs to the tumor sites and the number of monocytes differentiated into M2 macrophages (Dandekar, Kingaonkar, Dhabekar 2011).

2 PREVIOUS MYELOID CELLS-RELATED STUDIES

2.1 Myeloid Cell Profiles and Immunotherapy Resistance Mechanism

Kim et al. provide evidence that intrinsic tumor pathways and mutual regulation between the neutrophils and macrophages contribute to the development of dichotomous myeloid (Mollica Poeta, Massara, Capucetti, & Bonecchi 2019). The dichotomous distribution between the macrophages and neutrophils is observed by mouse breast cancer models, found out that breast cancer can be divided into 'hot tumors' (with rich immune cells) and 'cold tumors.' And the 'hot' tumor could be further divided into neutrophil-enriched subtypes (also known as the NES) and macrophage-enriched subtype (MES) group. The theory had been confirmed in human breast cancer by analyzing the TNBC database (Kim et al 2019).

Further, authors derived transcriptomic data sets from various murine syngeneic mammary tumor models and compared them to human breast cancer data (Kim et al 2019). Kim et al. demonstrate that human and murine cancers share many similarities. But it is hard to control the immune microenvironments with only one changing variable in clinical trials (Kim et al 2019).

When breast cancer of NES and MES were inoculated on both sides of mice, the different subtypes will maintain their neutrophil and macrophage frequencies, demonstrating the formation of different myeloid subtypes are caused by intrinsic tumor factors (Kim et al 2019). Thus, the internal tumor factors could contribute to myeloid cell profiles.

Some macrophages in different MES have high signaling pathways that promote immune suppression and promote tumor formation. In contrast, others have increased the expression of signaling pathways that promote the inflammatory response (Kim et al 2019). And an increase in monocytes is caused by the decrease in neutrophils for NES.

Moreover, the ICB therapy had been found to have a good effect on enriching MES in macrophages with high expression of promoting inflammatory responder signaling pathways. The ICB therapy has a moderate impact on the enrichment of MES in macrophages with increased expression of promoting suppression of immune signaling pathways but could improve the efficacy by combining CCR2KO and ICB therapy. And no effect on NES at all as the reduction of neutrophils hasn't enhanced the effectiveness of ICB therapy for NES (Kim et al 2019).

The immunotherapy resistance mechanisms show the importance of the immune microenvironment to tumor heterogeneity. And provide a potential route in improving the effectiveness of immunotherapy (Kim et al 2019).

2.2 Promote Macrophage Recruitment via the CXCL12/CXCR4 Pathway

CXCL12/CXCR4 pathway is one of the potential mechanisms to actively recruits abundant macrophages, especially pro-tumor M2 macrophages, that achieves the macrophage-enriched feature.

The mechanism of how TAMs, especially tumor-promoting M2 macrophages, migrate and accumulate in the OSCC via the interaction between CAF, CXCL12/ CXCR4 pathway, CSC, and M2 macrophages is demonstrated in a previous study (Li et al 2019). In the research conducted by Li et al., an in vivo test investigated the distribution and phenotype of TAM in OSCC. Overexpression of macrophages was observed in all OSCC samples compared to normal and dysplasia specimens. Besides, masses of these macrophages are pro-tumor M2 (Li et al 2019). Using the human monocytic cell line THP-1, resultant data shown that CAFs were most efficient at recruiting THP-1 monocytes. Among CAF-derived chemokines, the disparity between expressions in normal cells and CAF cells was the most significant for the CXCL12 chemokine (24 folds) (Li et al 2019). All data from chemotactic experiments suggested that CXCL12 was the predominant chemoattractant to drive monocyte

migration and recruitment (Li et al 2019). By labeling two subtypes of TAM, tumor-suppressive M1 and tumor-supportive M2, with distinct markers, the genotype of macrophages that were differentiated from THP-1 monocytes with the induction of CAF was identified as M2 macrophages (Li et al 2019). Besides, the cell viability assay demonstrated that M2 cells are crucial to promote proliferation and hinder apoptosis of Cal-27 cancer cells (Li et al 2019). By culturing Cal-27 cells with M2 macrophages, it was confirmed that polarized M2 cells induce OSCC to obtain the cancer stem cell-like characteristic. M2-treated Cal 27 cells were further confirmed that M2 macrophages had high resistance to the chemotherapy drug Vincristine (Li et al 2019). After conducting both transwell invasion assay and wound-healing assay to investigate the mobility of Cal 27 cells, the results revealed that M2 macrophages lead to expedited metastasis of OSCC via epithelial-mesenchymal transition (EMT) (Li et al 2019). In short, CXCL12/ CXCR4 pathway is critical in recruiting M2 macrophage polarization in OSCC, resulting in enhanced cancer cell proliferation, migration, and chemotherapy drug resistance.

2.3 Modified Labeling Method for Visualization and Quantification of MDSCs

Positron emission tomography (PET) is a nuclear medicine technique that measures the metabolism of cells in body tissues using tiny quantities of radioactive chemicals called radiopharmaceuticals to aid in the visualization of biochemical changes in the body (Positron Emission Tomography (PET). (n.d.). Thus, PET imaging is a powerful technique that enables the visualization and monitoring of myeloid cell distribution in TME (Hoffmann et al 2019). As mentioned above, myeloid-derived suppressor cells (MDSCs), a type of myeloid cells, are key participants within the tumor microenvironment (TME). Hoffman et al. applied an improved intracellular cell labeling approach to quantify in vitro-cultured MDSC motility in primary and metastatic cancers in vitro (Hoffmann et al 2019). Researchers labeled in vitro produced MDSCs from polymorphonuclear (PMN-) and monocytic (M-) subsets using a " ^{64}Cu-labeled 1,4,7-triazacyclononane-tri acetic acid (NOTA)-treated CD11b-specific monoclonal antibody (mAB)" (Hoffmann et al 2019). Subsequent to transferring them into primary and metastatic MMTV-PyMT breast cancer and B16/F10 melanoma mouse models respectively, PET and magnetic resonance images can be acquired for visualizing and quantifying MDSCs migrations (Hoffmann et al 2019). The researchers also indicate that the ^{64}Cu-NOTA-CD11b-mAB could be internalized within only 3 hours, resulting in moderately stable radiolabeling with minimal detrimental influence on cell survival and functionality. Furthermore, it was suggested by researchers that CD11b-specific mAB can simply adapt to label additional myeloid cells, including monocytes, macrophages, or neutrophils, for in vivo molecular imaging (Hoffmann et al 2019).

3 DISCUSSION

Besides OSCC, CXCL12/CXCR4 also impacts other types of cancers. Breast cancer remained the most frequent cancer among women globally, and it had been revealed that the CXCL12 and CXCR4 signaling has been implicated in practically every facet of breast cancer carcinogenesis (Zlotnik, Burkhardt, & Homey 2011). Chemokines are chemotactic cytokines and could be divided into different subgroups. The subgroup that interacts with their receptors could influence tumor growth and metastasis (Mollica Poeta et al, 2019b, Eckert et al 2018). Several specific chemokine receptors are found on both immune and tumor cells. And their presence on cancer cells could aid in cancer diagnosis (Jacquelot, Duong, Belz, Zitvogel 2018).

CXCR4 is a crucial signal in breast cancer metastasis as it expresses high levels of CXCR4 ligand CXCL12 in many organs, including liver, bones, lungs, and lymph nodes (Janssens, Struyf, & Proost, 2018, Koizumi, Hojo, Akashi, Yasumoto, & Saiki 2007, Balkwill 2004). By the presence of the CXCR4 positive cells in breast cancer patients' lymph nodes, the pDCs will secrete TNF, which induces CXCR4 expression in their body, producing a high expression of CXCL12 (Okuyama Kishima et al 2015). Besides, the CXCR4/CXCL12 pathway also plays a vital role in the prevention of lung metastasis. The CXCR2 ligands will recruit the CXCR2^{+} neutrophils into the TME, where they will interact with cancer cells and promote the expression of genes implicated in metastasis (Yu et al 2016).

As described above, the CXCL12/CXCR4 pathway has a considerable effect on tumor development, and therefore targeting the pathway could illustrate a potent method to create innovative therapy in cancer treatment. CXCR4 combines with its ligand CXCL12 and then becomes able to activate the downstream signaling pathway to boost cancer development. Among the current workable therapies

targeting on CXCL12/CXCR4 pathway, CXCR4 inhibitors are the major direction of massive research, because it is thought that CXCR4 antagonism can prevent cancer from growing (Zhou et al 2018).

Plerixafor, also called "AMD3100", is the only CXCR4 antagonist currently in clinical use (Zhou et al 2018). Plerixafor is a tiny bicyclam molecule with antiretroviral properties that could bind to CXCR4 (Zhou et al 2018). Since December 2008, it has been made available to non-lymphoma Hodgkin's (NHL) and multiple myeloma (MM) patients in the United States (Zhou et al 2018). The vital function for Plerixafor to prevent cancer development is generated by its ability to block the signaling pathway of CXCR4 after binding to CXCR4. However, this drug still has some deficiencies. Plerixafor lacks CXCR4 specificity. Research has demonstrated that Plerixafor only competes for CXCL12 binding to CXCR4 when high quantities of Plerixafor are present, indicating that Plerixafor is a low-affinity CXCR4 ligand. Plerixafor promotes CXCL12 binding to CXCR7 and triggers the CXCL12/CXCR7 signaling pathway (Kalatskaya 2009). Therefore, it is necessary to find other efficient treatment targeting on CXCL12/CXCR4 pathway.

4 POTENTIAL NOVEL TREATMENT

Owing to the lack of well-developed treatment targeting the CXCL12/CXCR4 interaction approved for clinical use, we herein propose a potential treatment that is based on the use of anti-CXCL12 monoclonal antibody (mAB) for OSCC. Fig.2 illustrates that CXCL12 mAB would bind to CXCR12; thus, preventing CXCL12 from interacting with CXCR4 receptor. It's expected to see reduced migration and expression of M2 macrophages in tumor sites with the anti-CXCL12 treatment.

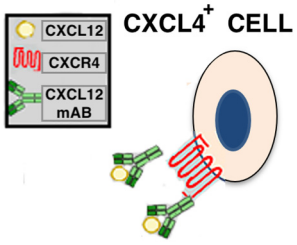

Figure 2. The CXCL12 mAB is expected to bind to CXCL12, which would prevent it from interacting with its receptor CXCR4 located on the cell surface. Figure modified from (Cancilla, Rettig & DiPersio 2020).

By using animal models, in vitro experiments are able to represent the whole organism with physiological relevance and the inherent complexity of a living system (Katt, Placone, Wong, Xu & Searson 2016). Due to the fact that mice are cheap, easy to breed, and biologically similar to humans, mice are the desired animal models to be used in this proposed design (Bryda 2013). OSCC-bearing Balb/c mice models can be established from the murine squamous cell carcinoma cell line, SCC7, which is indicated to result in stable syngeneic OSCC models in a previous study (Li 2020). Due to its ability to recognize CXCL12 in both human and mice models, monoclonal antibody (mAB) MAB350 (R&D Systems, Inc) is selected to test the hypothesis that neutralizing CXCL12 ligands is capable of reducing M2 macrophage recruitments and treating OSCC in the living organism (Human/Mouse CXCL12/SDF-1 antibody. 2020). The negative control is achieved with no injection of MAB350 into mice, while the experimental group is peritumoral injected with anti-CXCL12 antibodies. Peritumoral injection would prevent artificial injury to surrounding tissues by the needle (Yoshida 2018).

The effect of injected mAB can be identified and visualized via PET imaging. Tumor-associated macrophages with the specific pro-tumor M2 phenotype could be cultured in vitro following the instructions mentioned in Rey-Giraud et al.'s study. In brief, freshly isolated monocytes would be cultured in XVivo 10 media with M-CSF for six days to allow for the generation of monocyte-derived M2 macrophages (Rey-Giraud, Hafner & Ries 2012). The fact that CD11b is a common surface protein expressed on both human and murine macrophages makes CD11b a great target for radiolabeling (Dziennis et al 1995). The procedure to radiolabel in vitro-generated M2 macrophages is adapted from Sabrina et al. In short, M2 macrophages are incubated with [^{64}Cu]NOTA-αCD11b-mAb in PBS media for half an hour at 37°C (Hoffmann et al 2019). Radiolabeled M2 macrophages are then transferred into OSCC-bearing mice models for PET imaging.

For 2hr, 4hr, and 12hr after injection of anti-CXCL12 mAB, a PET scan would be acquired for each mice model (InveonTM user manual: Inveon scanners and inveon acquisition workplace 1.5 with service pack 1. 2011). By quantitatively analyzing and comparing the signal of the PET images, we might be able to examine the migration of labeled M2 macrophages to the OSCC tumor sites. Since the expression of M2 macrophages is confirmed to correlate with tumor progression positively, lower levels of M2 macrophages are expected in anti-

CXCL12 mAb-treated mice models (Li et al 2019). The negative control is estimated to have a much higher expression of M2 macrophages than all experimental models. Results that match our expectations could verify our hypothesis that potential cancer treatment targeting on CXCL12/CXCR4 pathway; specifically, the use of anti-CXCL12 antibodies is a feasible therapy.

5 CONCLUSIONS

Briefly, based on different characteristics of various myeloid cells and previous myeloid cells-related studies, the CXCL12/CXCR4 pathway was used as an entry point to identify viable therapies for this pathway, namely the use of Plerixafor (Righetti 2019, Kim et al 2019). Due to drawbacks of Plerixafor, it is necessary to continue searching for alternative therapeutic approaches targeting the CXCL12/CXCR4 pathway. Herein, a potential cancer treatment pathway is proposed: by developing CXCL12 inhibitors, the migration and expression of M2 macrophages at the tumor site are reduced. This treatment can be done as a further test in humans after the effects are confirmed in animal models.

REFERENCES

A., T. (n.d.). Immunology: What cells have a myeloid lineage and how are they identified? Cell Signaling Technology. Retrieved March 3, 2021, from https://blog.cellsignal.com/immunology-what-cells-have-a-myeloid-lineage-and-how-are-they-identified

A., T. (n.d.). Immunology: What cells have a myeloid lineage and how are they identified? Cell Signaling Technology. Retrieved August 28, 2021, from https://blog.cellsignal.com/immunology-what-cells-have-a-myeloid-lineage-and-how-are-they-identified

Akram, M., Iqbal, M., Daniyal, M., & Khan, A. U. (2017). Awareness and current knowledge of breast cancer. Biological research, 50(1), 33. https://doi.org/10.1186/s40659-017-0140-9

Balkwill, F. (2004). Cancer and the chemokine network. Nature Reviews Cancer, 4(7), 540–550. https://doi.org/10.1038/nrc1388

Breast cancer | world cancer research fund international. (2021, May 19). WCRF International. https://www.wcrf.org/dietandcancer/breast-cancer/#:%7E:text=Breast%20cancer%20is%20the%20most,per%20100%2C000%20in%20Northern%20America.

Brostjan, C., & Oehler, R. (2020). The role of neutrophil death in chronic inflammation and cancer. Cell Death Discovery, 6(1). https://doi.org/10.1038/s41420-020-0255-6

Bryda E. C. (2013). The Mighty Mouse: the impact of rodents on advances in biomedical research. Missouri medicine, 110(3), 207–211.

Cancer. (2021, March 3). World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cancer

Cancilla, D., Rettig, M. P., & DiPersio, J. F. (2020). Targeting CXCR4 in AML and ALL. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.01672

Dandekar, R. C., Kingaonkar, A. V., & Dhabekar, G. S. (2011). Role of macrophages in malignancy. Annals of maxillofacial surgery, 1(2), 150–154. https://doi.org/10.4103/2231-0746.92782

Davis, L. E., Shalin, S. C., & Tackett, A. J. (2019). Current state of melanoma diagnosis and treatment. Cancer biology & therapy, 20(11), 1366–1379. https://doi.org/10.1080/15384047.2019.1640032

Dziennis, S., Van Etten, R., Pahl, H., Morris, D., Rothstein, T., Blosch, C., Perlmutter, R., & Tenen, D. (1995). The CD11b promoter directs high-level expression of reporter genes in macrophages in transgenic mice [published erratum appears in Blood 1995 Apr 1;85(7):1983]. Blood, 85(2), 319–329. https://doi.org/10.1182/blood.v85.2.319.319

Eckert, F., Schilbach, K., Klumpp, L., Bardoscia, L., Sezgin, E. C., Schwab, M., Zips, D., & Huber, S. M. (2018). Potential role of CXCR4 targeting in the context of radiotherapy and immunotherapy of cancer. Frontiers in Immunology, 9. https://doi.org/10.3389/fimmu.2018.03018

Hoffmann, S. H. L., Reck, D. I., Maurer, A., Fehrenbacher, B., Sceneay, J. E., Poxleitner, M., Öz, H. H., Ehrlichmann, W., Reischl, G., Fuchs, K., Schaller, M., Hartl, D., Kneilling, M., Möller, A., Pichler, B. J., & Griessinger, C. M. (2019). Visualization and quantification of in vivo homing kinetics of myeloid-derived suppressor cells in primary and metastatic cancer. Theranostics, 9(20), 5869–5885. https://doi.org/10.7150/thno.33275

Human/Mouse CXCL12/SDF-1 antibody. (2020, August 25). R&D Systems. https://www.rndsystems.com/products/human-mouse-cxcl12-sdf-1-antibody-79018_mab350#product-details

InveonTM user manual: Inveon scanners and inveon acquisition workplace 1.5 with service pack 1. (2011). Siemens Medical Solutions USA, Inc. https://cancer.wisc.edu/research/wp-content/uploads/2018/02/inveon_iaw15_manual.pdf

Jacquelot, N., Duong, C. P. M., Belz, G. T., & Zitvogel, L. (2018). Targeting chemokines and chemokine receptors in melanoma and other cancers. Frontiers in Immunology, 9. https://doi.org/10.3389/fimmu.2018.02480

Janssens, R., Struyf, S., & Proost, P. (2018). Pathological roles of the homeostatic chemokine CXCL12. Cytokine & Growth Factor Reviews, 44, 51–68. https://doi.org/10.1016/j.cytogfr.2018.10.004

Kalatskaya, I., Berchiche, Y. A., Gravel, S., Limberg, B. J., Rosenbaum, J. S., & Heveker, N. (2009). AMD3100 is a CXCR7 ligand with allosteric agonist properties. Molecular pharmacology, 75(5), 1240–1247. https://doi.org/10.1124/mol.108.053389

Katt, M. E., Placone, A. L., Wong, A. D., Xu, Z. S., & Searson, P. C. (2016). In vitro tumor models: Advantages, disadvantages, variables, and selecting the right platform. Frontiers in Bioengineering and Biotechnology, 4. https://doi.org/10.3389/fbioe.2016.00012

Kim, I. S., Gao, Y., Welte, T., Wang, H., Liu, J., Janghorban, M., Sheng, K., Niu, Y., Goldstein, A., Zhao, N., Bado, I., Lo, H. C., Toneff, M. J., Nguyen, T., Bu, W., Jiang, W., Arnold, J., Gu, F., He, J., . . . Zhang, X. H. F. (2019). Immuno-subtyping of breast cancer reveals distinct myeloid cell profiles and immunotherapy resistance mechanisms. Nature Cell Biology, 21(9), 1113–1126. https://doi.org/10.1038/s41556-019-0373-7

Koizumi, K., Hojo, S., Akashi, T., Yasumoto, K., & Saiki, I. (2007). Chemokine receptors in cancer metastasis and cancer cell-derived chemokines in host immune response. Cancer Science, 98(11), 1652–1658. https://doi.org/10.1111/j.1349-7006.2007.00606.x

Li, Q., Dong, H., Yang, G., Song, Y., Mou, Y., & Ni, Y. (2020). Mouse Tumor-Bearing models as preclinical study platforms for oral squamous cell carcinoma. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.00212

Li, X., Bu, W., Meng, L., Liu, X., Wang, S., Jiang, L., Ren, M., Fan, Y., & Sun, H. (2019). CXCL12/CXCR4 pathway orchestrates CSC-like properties by CAF recruited tumor associated macrophage in OSCC. Experimental Cell Research, 378(2), 131–138. https://doi.org/10.1016/j.yexcr.2019.03.013

Liu, T., Han, C., Wang, S., Fang, P., Ma, Z., Xu, L., & Yin, R. (2019). Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. Journal of Hematology & Oncology, 12(1). https://doi.org/10.1186/s13045-019-0770-1

Lv, M., Wang, K., & Huang, X. J. (2019). Myeloid-derived suppressor cells inhematological malignancies: friends or foes. Journal of Hematology & Oncology, 12(1). https://doi.org/10.1186/s13045-019-0797-3

Mollica Poeta, V., Massara, M., Capucetti, A., & Bonecchi, R. (2019). Chemokines and chemokine receptors: New targets for cancer immunotherapy. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.00379

Mollica Poeta, V., Massara, M., Capucetti, A., & Bonecchi, R. (2019b). Chemokines and chemokine receptors: New targets for cancer immunotherapy. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.00379

Morris, S. Y. (2018, September 29). Understanding neutrophils: Function, counts, and more. Healthline. https://www.healthline.com/health/neutrophils

NCI Dictionary of Cancer Terms. (n.d.). National Cancer Institute. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/five-year-survival-rate

Okuyama Kishima, M., Oliveira, C. E. C. D., Banin-Hirata, B. K., Losi-Guembarovski, R., Brajão De Oliveira, K., Amarante, M. K., & Watanabe, M. A. E. (2015). Immunohistochemical expression of CXCR4 on breast cancer and its clinical significance. Analytical Cellular Pathology, 2015, 1–6. https://doi.org/10.1155/2015/891020

Ping, Q., Yan, R., Cheng, X., Wang, W., Zhong, Y., Hou, Z., Shi, Y., Wang, C., & Li, R. (2021). Cancer-associated fibroblasts: Overview, progress, challenges, and directions. Cancer Gene Therapy. Published. https://doi.org/10.1038/s41417-021-00318-4

Positron Emission Tomography (PET). (n.d.). Johns Hopkins Medicine. https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/positron-emission-tomography-pet

Rey-Giraud, F., Hafner, M., & Ries, C. H. (2012). In vitro generation of monocyte-derived macrophages under serum-free conditions improves their tumor promoting functions. PloS one, 7(8), e42656. https://doi.org/10.1371/journal.pone.0042656

Righetti, A., Giulietti, M., ŠAbanović, B., Occhipinti, G., Principato, G., & Piva, F. (2019). CXCL12 and its isoforms: Different roles in pancreatic cancer? Journal of Oncology, 2019, 1–13. https://doi.org/10.1155/2019/9681698

Schmid, M. C., & Varner, J. A. (2010). Myeloid cells in the tumor microenvironment: Modulation of tumor angiogenesis and tumor inflammation. Journal of Oncology, 2010, 1–10. https://doi.org/10.1155/2010/201026

Taghavi, N., & Yazdi, I. (2015). Prognostic factors of survival rate in oral squamous cell carcinoma: clinical, histologic, genetic and molecular concepts. Archives of Iranian medicine, 18(5), 314–319.

Veglia, F., Perego, M., & Gabrilovich, D. (2018). Myeloid-derived suppressor cells coming of age. Nature Immunology, 19(2), 108–119. https://doi.org/10.1038/s41590-017-0022-x

Wright stain. (2020, January 5). Lab Tests Guide. https://www.labtestsguide.com/wright-stain

Yoshida, H., Yoshimura, H., Matsuda, S., Ryoke, T., Kiyoshima, T., Kobayashi, M., & Sano, K. (2018). Effects of peritumoral bevacizumab injection against oral squamous cell carcinoma in a nude mouse xenograft model: A preliminary study. Oncology Letters. Published. https://doi.org/10.3892/ol.2018.8399

Yu, P. F., Huang, Y., Xu, C. L., Lin, L. Y., Han, Y. Y., Sun, W. H., Hu, G. H., Rabson, A. B., Wang, Y., & Shi, Y. F. (2016). Downregulation of CXCL12 in mesenchymal stromal cells by TGFβ promotes breast cancer metastasis. Oncogene, 36(6), 840–849. https://doi.org/10.1038/onc.2016.252

Zhou, Y., Cao, H. B., Li, W. J., & Zhao, L. (2018). The CXCL12 (SDF-1)/CXCR4 chemokine axis: Oncogenic properties, molecular targeting, and synthetic and natural product CXCR4 inhibitors for cancer therapy.

Chinese Journal of Natural Medicines, 16(11), 801–810. https://doi.org/10.1016/s1875-5364(18)30122-5

Zlotnik, A., Burkhardt, A. M., & Homey, B. (2011). Homeostatic chemokine receptors and organ-specific metastasis. Nature Reviews Immunology, 11(9), 597–606. https://doi.org/10.1038/nri3049