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The current paradigm and challenges ahead for disseminated tumor cell dormancy

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17 **ABSTRACT:**

18
19 Disseminated tumor cells (DTCs) are known to enter a state of dormancy, which is achieved
20 via growth arrest of DTCs and/or a form of population equilibrium state, strongly influenced by the
21 organ microenvironment. During this time, expansion of residual disseminated cancer is paused and
22 DTCs survive to fuel relapse, sometimes decades later. This notion has opened a new window of
23 opportunity to intervene and prevent relapse. Here we review recent data that have further
24 augmented our understanding of cancer dormancy and discuss how this is leading to new strategies
25 to monitor and target dormant cancer.

26 INTRODUCTION

27 In recent years our understanding of metastasis biology and cancer cell dormancy has
28 advanced considerably. Current knowledge of how cancer dormancy proceeds has stemmed from
29 pioneering work on angiogenesis¹⁻³, immune regulation of equilibrium states and antibody signaling⁴⁻
30 ¹³, and on how micro-environmental and signaling mechanisms control cellular dormancy through
31 growth arrest programs^{14,15}. Key findings based on this body of work proposed that dormant cells
32 might evade anti-proliferative therapies in a passive manner¹⁶. Since then, the field has expanded
33 significantly revealing the importance of the tissue microenvironment in driving dormancy and
34 dormant cell reactivation. Developmental cues that contribute to tissue homeostasis by controlling
35 quiescence of normal adult stem cells, and various niche cells of different organs have been shown to
36 also drive dormancy (¹⁷⁻¹⁹). Dormant cancer cells were also found to regulate active signaling
37 mechanisms of adaptation and survival to therapies²⁰⁻²⁴, demonstrating that dormant tumor cells do
38 not merely survive chemotherapeutic drug insults in a passive manner. These findings have
39 strengthened the notion that dormant DTCs persist over long periods of time by co-opting conserved
40 growth arrest and survival mechanisms that are active during development and in adult tissues.

41 In this Perspective article, we focus on recent, primarily *in vivo* and human data and discuss
42 how they inform evolving concepts regarding tumor dormancy, including the active role of the
43 microenvironment.

45 DISCUSSING DEFINITIONS

46 The cancer dormancy field has worked based on three definitions that are not necessarily
47 mutually exclusive and may be complementary. These have led to the working hypothesis that
48 asymptomatic minimal residual disease (MRD) can be defined and explained by three potential
49 scenarios: 1) angiogenic dormancy, an impaired angiogenic response maintaining tumor mass
50 constant in size by balancing proliferation and cell death¹⁻³; 2) immune-mediated dormancy, in which
51 proliferative tumor cell populations are constantly trimmed by cytotoxic immune cell responses that
52 also maintain an equilibrium between cell death and proliferation⁴⁻¹³; and 3) cellular dormancy, in
53 which solitary DTCs or small cell clusters enter a prolonged growth arrest with no increase in cell
54 death^{19,24-27}.

55 Equilibrium states, as described in the angiogenic dormancy program³, have long been
56 proposed as a defining feature of dormant tumor cell mass dynamics, with evidence accumulating
57 since the 1990's. This concept and the relevant literature has been reviewed extensively

58 elsewhere^{18,26,28}. The immune equilibrium hypothesis suggests that immune surveillance keeps
59 proliferating cell populations in check during MRD through T cell-mediated killing or anti-idiotypic
60 antibody networks that cause growth suppression in mouse lymphoma, and has also been reviewed
61 previously^{26,29}. Recent studies that focused more on cellular dormancy and its relationship with
62 immune cells, indicate that quiescent DTCs are in fact evading CD8⁺ T cell and natural killer (NK)-cell
63 mediated detection and clearing^{27,30}. Thus, as proposed in an integrated scenario²⁶, quiescent DTCs
64 may evade immune detection, but as they switch into proliferation they may be maintained in
65 equilibrium by immune cytotoxic responses, showing how these two processes can be
66 complementary. To our knowledge there are no available studies showing that dormant solitary DTCs
67 that become reactivated are then kept under tumor mass dormancy by an angiogenic switch failure,
68 but this possibility has also been proposed²⁶. The large body of literature discussed in this article has
69 independently discovered that dormant cancer cell populations consist of single solitary DTC or small
70 DTC clusters of 10-20 cells^{18,23,27,31-36}. These DTCs are able to enter a reversible growth arrest or
71 quiescence state, supporting that this may be a common feature of solitary dormant cancer cells.

72 Whether all the above scenarios can co-exist in patients remains an open question. The
73 tissues that are sampled for further study may also bias the identification of the mechanisms involved.
74 For example, several dormancy studies that complement the study of human DTCs from bone
75 marrow with mouse models seem to point to quiescence as a feature of solitary DTCs^{17,19,26,37-39}. This
76 process appears to be governed by micro-environmental cues, resembling the lifelong quiescence
77 program active in dormant adult stem cells, including in hematopoietic, muscle, neural and hair follicle
78 stem cells^{17,26,37,38,40,41} (**Figure 1**). However, mechanisms of senescence or differentiation have also
79 been proposed to underlie DTC dormancy^{18,42,43}. A confounding factor is that growth arrest programs
80 during quiescence, senescence and differentiation exhibit substantial overlap with respect to the
81 regulation of the cell cycle machinery (**Figure 1**). Nevertheless, senescence is by definition
82 irreversible and regulators of this program, such as p16 and p53, are commonly mutated or silenced
83 early in tumorigenesis⁴⁴. Additionally, senescence leads to the clearing of senescent cells by innate
84 immune cells⁴⁵, which has so far not been documented for potentially “senescent” DTCs. Importantly,
85 several studies have documented that DTCs activate variations of pluripotency programs and a
86 certain degree of chromatin remodeling linked to enhanced epigenetic plasticity (⁴⁶⁻⁵¹ and section
87 below). On the contrary, senescent cells commonly show highly repressive heterochromatin, which
88 limits plasticity⁵² (**Figure 1**). However, the possibility that a hybrid state of senescence programs is
89 activated by micro-environmental cues⁴³ or therapeutic stress⁵³ cannot be ruled out.

90 Indeed, a key attribute of dormant DTCs is their ability to retain a high degree of epigenetic
91 and transcriptional plasticity and to reactivate different developmental programs to arrest growth and
92 survive (see^{17,54} and section below). This high level of plasticity that enables cancer cells to employ
93 various regulatory programs to induce a reversible growth arrest, thereby ensuring persistence and
94 conferring an adaptive advantage, may be the true defining feature of dormancy. Thus, two key
95 elements emerge as necessary to better understand DTC dormancy. One is to better define the
96 identified programs functionally and molecularly, to understand how they resemble quiescence,
97 senescence or differentiation and how they intersect with angiogenic dormancy and immune-
98 mediated equilibriums. The second is to define which of the programs identified in models are
99 functionally relevant in patients.

101 **TARGET ORGAN MICROENVIRONMENTS**

102 The tumor microenvironment consists of cellular and extracellular components that surround
103 cancer cells. In the primary tumor site it can represent half or more of the tumor mass. However, in
104 target organs where solitary or small clusters of DTCs reside following dissemination, DTCs are
105 initially a minor constituent of the tissue. Thus, how they interpret this new microenvironment and how
106 the signals encoded in these initially normal tissue niches impact cancer dormancy induction,
107 maintenance and escape are important questions.

109 **BONE MARROW NICHES.** Animal and human biopsy studies support the notion that DTCs are
110 likely reaching multiple organs but only survive and eventually grow in specific tissues and in a cancer
111 specific manner. Many studies have focused on the bone marrow (BM) as cancer cells can persist in
112 this site for years without growing and the detection of BM-DTCs is commonly a poor prognosis
113 marker⁵⁵⁻⁵⁸. In the BM, osteoblasts have been suggested to induce solid tumor DTC dormancy⁵⁹, with
114 osteoclasts being involved in the escape from dormancy during the process of osteolytic bone
115 metastases⁶⁰ (**Figure 2**). The role of osteoblasts in dormancy and bone metastasis is however more
116 complex. Whereas the endosteal niche and osteoblasts can promote cancer cell dormancy⁵⁹,
117 remodeling of this niche could cause reactivation or promote survival of cancer cells⁶¹. For example,
118 cancer cells depend on physical interactions with osteogenic cells through gap junctions to increase
119 their intracellular calcium levels and therefore the osteogenic niche, which includes osteoblasts,
120 serves as a calcium reservoir for cancer cells to form micro-metastases⁶². Additionally, changes in
121 osteoblast phenotype, such as acquisition of an aged or senescent phenotype could fuel

122 metastasis⁶³. Recently, significant work has focused on vascular niches that induce sustained DTC
123 dormancy in the BM^{23,31}, brain³⁵ and lungs³⁴ (**Figure 2**). BM vascular niches were shown to protect
124 DTCs from chemotherapy through an integrin-mediated interaction between DTCs and molecules
125 within the perivascular niches, including VWF and VCAM1²³. Disrupting these interactions with
126 integrin blocking antibodies results in a reduction in DTC burden and prevention of bone metastasis.
127 Importantly, although chemo-protection by the vascular niche appears to be cell cycle-independent,
128 inhibiting integrin $\beta 1$ and/or integrin $\alpha v \beta 3$ enhanced chemotherapeutic response. How chemotherapy
129 is killing non-dividing DTCs that have ablated integrin signaling is still unknown. Perhaps cancer cells
130 become sensitized during the first divisions following their exit from the single cell state. In the brain,
131 DTCs breach the blood–brain barrier and adhere to brain capillaries in mice³⁵. The position and
132 morphology of DTCs is reminiscent of pericytes as these cells spread on brain capillaries between
133 pericytes and capillary surfaces, migrated along the vessels and remained dormant for long periods³⁴.
134 Although the mechanism of dormancy was not explored in this study, L1CAM on DTCs was found to
135 mediate access to the perivascular niche and drive transcriptional programs that awakened dormant
136 DTCs, leading to metastasis initiation. If the reactivation of DTCs was caused by their pericyte-like
137 behavior or changes in the niche itself is unclear. However, changes in the niche, as elicited by tip
138 endothelia cells, are known to cause reactivation, whereas stalk endothelial cells promote
139 dormancy⁶⁴. Similarly, endothelial cell expression of L1CAM ligands allow L1CAM+ DTCs to engage
140 proliferative programs, but it is possible that if the endothelium or other perivascular cells do not
141 express such ligands, L1CAM+ DTCs may not be able to overcome dormancy signals. Therefore, the
142 perivascular niche could induce or maintain DTC dormancy in a context-dependent manner.

143 Elegant, conditional tissue-specific, knockout mouse models and bone transplantation
144 approaches revealed that perivascular CXCL12-expressing Prx1+ mesenchymal stem cells (MSCs)
145 maintained leukemic stem cells (LSCs) quiescent and treatment-resistant in the BM⁶⁵. Targeted
146 deletion of CXCL12 in MSCs reduced normal hematopoietic stem cell (HSCs) numbers, but promoted
147 LSC expansion by increasing self-renewing cell divisions, with LSCs being eliminated by tyrosine
148 kinase inhibitor (TKI) treatment. Moreover, chronic myeloid leukemia (CML) cells induced a reduction
149 in CXCL12-expressing MSCs and increase in CXCL12-expressing endothelial cells, alterations that
150 may provide a competitive advantage to LSCs over HSCs (**Figure 3**). This work highlights how the
151 perivascular niche may allow targeting DTC-niche interactions in solid and hematopoietic cancer cell
152 dormancy to interfere with dormant DTCs that cause recurrence.

153 Open questions are whether DTCs move stochastically or are actively recruited into a

154 perivascular niche where they enter dormancy, or whether these are simply the niches that promote
155 survival and quiescence. A recent study showed that similar to benign and malignant hematopoietic
156 cells⁶⁶, breast cancer DTCs enter the BM through the sinusoidal vasculature that expresses the
157 inflammatory molecules E-selectin and SDF-1, both in mice and human samples⁶⁷ (**Figure 3**). Mice
158 treated with an E-selectin inhibitor had fewer DTCs in the BM, arguing for decreased homing. In
159 another model⁶⁸, E-selectin was identified as a pro-metastatic receptor of the bone vascular niche,
160 with its pharmacological inhibition increasing survival and decreasing bone metastasis-associated
161 bone degradation. Thus, rather than homing, this study suggested a growth-promoting effect for E-
162 selectin that was Wnt-dependent and bone specific. The differences between the specific findings of
163 these two studies are unclear, but may depend on differences in the models of BM colonization used
164 and in the timing and type of endpoints assessed.

165 BM colonization and metastasis are common late relapse occurrences in hormone- (and thus
166 microenvironment-) regulated ER+ breast cancer. A recent study identified MSK1 as a potential
167 regulator of ER+ breast cancer dormancy⁶⁹. MSK1 depletion was shown to lead to the epigenetic
168 downregulation of genes controlling luminal cell fate, such as FOXA1 and GATA3, and contributing to
169 an increased metastasis-initiating potential. This effect was also observed when p38 signaling was
170 chemically inhibited, as MSK1 is a target of the p38 kinase⁶⁹, reproducing the role of this pathway in
171 dormancy¹⁴. Anti-estrogen therapy is an effective therapy in human breast cancer^{70,71}, but does not
172 eradicate the disease in many patients⁷¹. Whether tamoxifen activates an MSK1-p38 α (or other)
173 signaling mechanism for dormancy is not clear, but understanding whether anti-estrogen therapies
174 are working through dormancy induction may be illuminating. Interestingly, dormant cell populations
175 were recently shown to be prevalent during ER α targeting⁷¹. A similar question was explored in
176 humans⁷² through the sequential analysis of samples from patients undergoing extended neoadjuvant
177 hormonal treatment. “Dormant” tumors (i.e. tumors from patients with tumor size reduction and no
178 progression after long post-treatment periods of time) displayed enhanced DNA methylation and a
179 more repressive chromatin state, whereas resistant tumors displayed the opposite profile. Although
180 these data are not directly derived from DTCs they may provide insights into the biomarkers to look
181 for in DTCs or stroma in the adjuvant setting, and determine if anti-estrogens are inducing dormancy
182 of residual DTCs. The BM is also a common site for dormancy of prostate cancer cells⁷³. Wnt5a
183 delivered systemically in a prostate cancer model of metastasis induced a dormancy-like
184 phenotype in cancer cells and a reduction in bone metastasis, potentially *via* ROR2 signaling⁷². This
185 treatment also revealed that Wnt5a favored chemoresistance to docetaxel. Additional analyses may

186 be needed to reveal whether these mechanisms are active in prostate cancer DTCs in the BM.
187 Interestingly, Wnt5a has also been linked to a senescence- or dormancy-like phenotype in
188 melanoma⁷⁴.

189 **INFLAMMATION AND ECM REMODELING.** Given that dormancy is a reversible state regulated by
190 the microenvironment, it is necessary to determine the microenvironmental changes that could trigger
191 awakening from dormancy (**Figure 2**). Bacterial lipopolysaccharide- (LPS) or tobacco smoke-induced
192 lung inflammation was recently shown to activate neutrophils to form neutrophil extracellular DNA
193 traps (NETs)³². NETs were found to trigger dormant DTC awakening through the release of neutrophil
194 elastase and matrix metalloprotease 9, enzymes that promote the laminin-111- and $\alpha3\beta1$ -integrin-
195 dependent activation of a signaling axis involving the FAK, ERK and MLCK kinases, and ultimately
196 the activation of the YAP transcriptional regulator. Interestingly, to fully awaken DTCs, loss of
197 perivascular TSP-1 was also required. Metastatic breast cancer cells can themselves induce
198 NETs^{32,75}, raising the possibility that they may be creating a positive pro-inflammatory proliferative
199 loop. Surgical stress was also shown to trigger DTC awakening through an increase in NET
200 formation, in a liver ischemia-reperfusion mouse model⁷⁶. Consistently, in a cohort of patients
201 undergoing attempted curative liver resection for metastatic colorectal cancer, a correlation was
202 observed between increased post-operative NET formation and reduction in disease-free survival⁷⁶.
203 In a model of pancreatic ductal adenocarcinoma (PDAC) the TRAIL-R2 pathway was also
204 suggested to lead to liver inflammation and awakening of dormant DTCs during tumor resection⁷⁷.
205 Thus, it is possible that tissues that remain homeostatic may maintain DTC dormancy, whereas
206 changes, such as inflammation, that alter the homeostatic balance (for example through NETs and
207 ECM remodeling) may trigger awakening from DTC dormancy.

208 An important question is the link between aging and metastatic relapse and the age- and
209 microenvironment-related signals that may regulate DTC dormancy and reactivation. Recently, in an
210 aging mouse model, metastatic cell lines were shown to be highly proliferative in aged BM, with
211 dormant DTC frequencies declining in aged mice as opposed to young mice⁷⁸. Mechanistic analysis
212 revealed that several inflammatory cytokines known to promote cell proliferation (including
213 interleukins 1b, 6, 27, 1F9, and factors such as CCL4/5 and Tnfsf14) were upregulated in bones of
214 aged mice, whereas proposed quiescence-inducing factors (including BMP4/6/7, Kitl, TGF β 2, Thbs2,
215 Dkk1/3) were downregulated⁷⁸ (**Table I**). ECM remodeling may also stimulate metastasis and
216 perhaps dormancy awakening, as revealed by changes in age related remodeling of the collagen
217 matrix that stimulated metastasis in melanoma⁷⁹⁻⁸¹.

218 The concept of reversion of the malignant phenotype by normalizing the microenvironment is
219 not new⁸²⁻⁸⁵ and supports that –in the appropriate context— cancer cells could remodel their
220 epigenetic programs to silence mutated genomes. Axolotl embryos have been shown to fully
221 reprogram malignant breast cancer cells into quiescence by inhibiting the ERK1/2-mediated induction
222 of p27 and reducing JUN- and Rb-dependent signaling⁸⁶. These reprogrammed breast cancer cells
223 also displayed a repressive chromatin state, previously reported in retinoic acid-induced dormancy⁴⁶.
224 Although the mechanisms activated by the axolotl extract were not identified, they reveal how
225 microenvironment signals can epigenetically silence malignancy traits.

226 These studies expand our understanding of how homeostasis of target organ
227 microenvironments, may maintain dormancy, likely governed by adult stem cell quiescence niches.
228 They also support that alterations during time (in this case the organism's age) or perturbations that
229 alter target organ microenvironments, such as inflammation and ECM remodeling, can control
230 dormancy. A key question is how robust the dormancy-inducing microenvironments are. Given that
231 for example in breast cancer, relapse occurring more than 5 years after surgery is mostly stochastic
232 and can be delayed for long periods, it is possible that multiple homeostatic barriers need to be
233 eliminated for DTCs to awaken. Although patients will encounter situations that may activate
234 inflammatory processes many times in life, many do not show immediate relapse.

235 **AUTOPHAGY AND METABOLISM.** Another important but poorly explored mechanism in dormancy
236 biology is metabolic plasticity. For example, unfolded protein response (UPR) pathways and HIF1 α
237 signaling, which are intimately linked to metabolic changes, can enable residual cancer cells to
238 persist and evade chemotherapy⁸⁷. Recently, glycerol-3-phosphate dehydrogenase 1 (GPD1) was
239 shown⁸⁸ to marked slow-cycling brain tumor stem cells with the ability to repopulate the tumor after
240 temozolomide (TMZ) therapy in glioblastoma mouse models. These GPD1+ cells were present in
241 tumor margins, suggesting that they might escape resection and therapy. However, whereas GPD1+
242 cells were mostly negative for proliferation markers, they were the cells able to repopulate the lesions
243 after therapy. This suggests that GPD1 is involved in metabolic plasticity of slow-cycling cells that
244 mediates reactivation but does not regulate long-term dormancy.

245 Autophagy is intimately linked to stress signaling and metabolic changes, as it is a source of
246 metabolites for energy production but also a way to protect from proteotoxicity. Dormant cancer cells
247 were shown to be more autophagic than their proliferating counterparts, thus allowing them to survive
248 in a quiescent state⁸⁹. Importantly, inhibiting autophagy specifically blocked survival of dormant cells
249 in the lung in an ATG7-dependent manner⁹⁰. A separate study recently showed that 6-phosphofructo-

250 2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) is linked to breast cancer metastatic relapse.
251 Interestingly, PFKFB3 was downregulated by the onset of autophagy, which coincided with dormancy
252 induction. Unlike an earlier study⁸⁹, but consistent with other work reporting that autophagy may be
253 inducing dormancy^{18,91}, this paper showed that autophagy inhibition restored PFKFB3 expression,
254 leading to reactivation from dormancy. When autophagy functions only as a survival pathway and
255 when it coordinates induction and maintenance of dormancy remains unclear. Elucidating this point is
256 important because the second possibility supports that targeting autophagy could have deleterious
257 effects for patients by causing reactivation from dormancy.

258 **IMMUNE SYSTEM AND DTC DORMANCY.** Major advances in immuno-oncology have fundamentally
259 altered our understanding of the relationship between the adaptive immune system and cancer cells,
260 leading to the current breakthroughs in immunotherapies. A key question is whether dormant DTCs
261 are restrained from expanding by an immune equilibrium mechanism and/or whether dormant DTCs
262 evade immune recognition (**Figure 2**). Pioneering studies on carcinogenesis and disseminated
263 cancer have provided support for a role of immune equilibrium as a mechanism of dormancy^{13,92}.
264 Similarly studies on a B-cell lymphoma that establishes dormancy through a vaccination protocol
265 revealed important components on how dormancy could be induced and maintained by CD8+ T cells
266 and IFN γ signaling⁶. Additionally, immune evasion was shown to allow the persistence of dormant
267 leukemic cells in mouse acute myeloid leukemia (AML) through the upregulation of B7-H1 (PD-L1)
268 and the CTLA-4 ligand B7.1, but without involving MHC-I decrease⁹³. More recently, it was shown
269 that mice vaccinated against PDAC cells, were protected against rechallenge with the same PDAC
270 cells injected to colonize the mice livers, as expected³⁰. However, although the immune system
271 eradicated most cancer cells, persistent single DTCs were detected. The immune evasive DTCs were
272 quiescent, did not express E-cadherin and upregulated the UPR, a pathway previously linked also to
273 dormant cancer cell drug resistance²². The UPR was found to downregulate MHC class I, reducing
274 antigen presentation and rendering dormant DTCs invisible to CD8+ T cells. Although the molecular
275 mechanisms differ, both leukemic and epithelial cancer cells that enter dormancy appear to find ways
276 to escape immune detection. As proposed earlier, immune evasion by dormant DTCs may simply be
277 reflect the hijacking of developmental or tissue homeostasis mechanisms. For example, quiescent
278 hair follicle stem cells were shown to evade CD8+ T cell immune recognition⁹⁴. It would be interesting
279 to test whether the signals that induce dormancy discussed in this article (**Table I**) modulate the
280 expression of key immune regulatory molecules such as MHC-I, PD-L1 and/or CTLA4, thereby
281 influencing immune detection. Dormant lung cancer and HER2+ breast cancer DTCs were also

282 shown to evade NK cell detection and to only be susceptible to eradication by NK cells following
283 escape from dormancy²⁷. Although these studies support that DTCs are NK-evasive during
284 quiescence, they only provide partial insight into their immune regulation as they were performed in
285 nude mice. Unlike these papers, the immune system of mice carrying the EMT6 syngeneic triple-
286 negative breast cancer model was shown to be able to fully eradicate dormant and proliferative
287 DTCs, suggesting their vulnerability to immune recognition⁹⁵. In contrast, a separate study in immune
288 competent mice⁹⁶ showed that D2A1 breast cancer cells were kept from forming metastases by
289 immune-mediated killing leading to an equilibrium that produced population dormancy. However,
290 when mice underwent sham surgery, the injury-associated inflammation and myeloid cell mobilization
291 led to immune suppression with the cancer cell population progressing to metastasis. Studies where
292 no exogenous antigens were used⁹⁵, may be relevant to understand how triple-negative breast
293 cancer patients that survive beyond 5-8 years post-surgery never relapse with metastasis. It would
294 also be interesting to determine whether primary tumor removal, as done routinely in patients, would
295 yield the same results as sham surgery in mice⁹⁶. Studies such as these, that formally interrogated
296 DTC biology and immune recognition, raise new questions related to DTC dormancy and immune-
297 therapies.

299 **COMMONALITIES IN LEUKEMIA AND DTC DORMANCY**

300 The HSC and leukemia fields have made significant advances in deciphering how specific
301 niches control HSC and leukemic stem cell (LSC) dormancy. At steady state, the BM niche is a tightly
302 controlled microenvironment regulating proliferation, self-renewal, differentiation and migration of
303 HSCs³⁷. HSCs and DTCs in the BM appear to be cell-cycle arrested. Moreover, these two types of
304 cells have been proposed to occupy the same BM niches and to respond to the microenvironment
305 cues similarly^{17,73} (**Figure 3**). Both dormant HSCs and DTCs appear to adopt quiescence and
306 survival mechanisms that enable them to avoid apoptosis and/or resist therapies. However, dormant
307 solid cancer cells share characteristics with embryonic and adult stem cells, such as upregulation of
308 pluripotency genes NR2F1, SOX9, SOX2, OCT4 and NANOG^{17,27,46,48,97}. This may be a distinction
309 between quiescence programs in HSCs and DTCs, where the latter cells may adopt developmental
310 programs that provide additional cell plasticity and less ability to commit to differentiation states.
311 However, other similarities are clear. For example, adult stem cell niches have also been shown to be
312 immune-protected sites⁹⁴, which may be advantageous for DTCs. Adult stem cells and quiescent
313 DTCs may also show downregulation of antigen presentation^{30,94}, supporting that escaping immune

314 surveillance may also be a common attribute. Another example involves the FBXW7 ubiquitin ligase.
315 inhibition of which was shown to prevent quiescence of LSCs⁹⁸ and to be involved in quiescence of
316 lung adenocarcinoma cells⁹⁹ and awakening and subsequent chemotherapy-mediated killing of breast
317 cancer DTCs¹⁰⁰. These data suggest that some quiescence/dormancy mechanisms may be shared
318 by LSCs and DTCs, thus providing novel potential anti-cancer targets to explore experimentally.
319 Importantly, the notion of awakening LSCs might work in some cases given the unique oncogene
320 addiction pathways of these cells⁶⁵, but in solid cancers, awakening dormant cells may be too risky
321 given that chemotherapy is not uniformly effective and responses may vary depending on the organ
322 in which metastasis grow.

323 Hematopoietic malignancies may also inform on cancer cell heterogeneity during dormancy.
324 Longitudinal BM sampling from patients to decipher clonal evolution from pre-leukemia to established
325 acute myeloid leukemia, revealed that some rare, likely dormant, leukemic cell sub-clones were
326 already present at diagnosis and were resistant to therapy, leading to relapse and highlighting the
327 importance of understanding the dynamic of LSC heterogeneity¹⁰¹. Similar studies on solid cancer
328 DTCs such as melanoma, breast and prostate cancer in patients with M0 stage (no evidence of
329 metastasis at diagnosis/surgery) could identify dormant DTCs that could be studied and targeted
330 early on. A single cell transcriptomic analysis of BM leukemic cells revealed that a specific sub-
331 fraction among the heterogeneous population of LSCs, characterized by stem cell and quiescence
332 signatures, survived in the BM environment of asymptomatic chronic myeloid leukemia (CML)
333 patients under TKI treatment, serving as a reservoir for the emergence of resistant cells¹⁰².
334 Treatment-induced quiescence of residual LSCs in this setting was demonstrated to rely on the
335 activation of Jak2-Stat3 signaling dependent on non-canonical BMP4 signaling, with the ligand
336 delivered by surrounding mesenchymal cells¹⁰³. Dual targeting of BMP and Jak2 was efficient in
337 reversing this TKI-dependent induced quiescence of the BMPR1B+ LSC sub-fraction adherent to
338 stroma and allowed them to re-enter a differentiation process¹⁰³. Such a strategy might contribute to
339 eliminating the reservoir of dormant LSCs.

340 Another study on treatment-induced quiescence in CML showed that the miR-126 microRNA
341 from endothelial or leukemic cells induces quiescence of CML-LSC¹⁰⁴. Interestingly, BCR-Abl is
342 known to inhibit the biogenesis of miR-126 and BCR-ABL inhibition restored miR-126 expression in
343 the cancer cells, leading to a quiescent LSC pool with enhanced survival. Moreover, combining BCR-
344 ABL inhibition and miR-126 downregulation resulted in LSC elimination supporting that this microRNA
345 may also coordinate survival during quiescence. Assessing if dormant DTCs from solid cancers are

346 regulated by similar mechanisms when using targeted therapies (e.g., anti-estrogens, anti-androgen
347 receptor, anti-HER2 or other kinases) could reveal novel cancer cell intrinsic and/or micro-
348 environmentally-induced mechanisms of DTC quiescence and survival triggered by therapeutic
349 interventions.

350 There is growing evidence that the niche influences cancer cells, but also that leukemic cells
351 can modulate their host BM microenvironment to survive and expand. For example, AML cells can
352 remodel the vascular organization of the endosteal niche, thereby altering the number of stromal
353 cells¹⁰⁵ and rendering the niche less supportive of normal HSCs and their quiescence. Additionally,
354 vascular permeability can be altered through the enhanced production of nitric oxide by endothelial
355 cells¹⁰⁶. Additional studies have highlighted the role of AML-derived extracellular vesicles in the
356 suppression of normal hematopoiesis by inhibiting protein synthesis¹⁰⁷ through the internalization of
357 miR-1246 in normal HSC or by inducing the expression of DKK1, a suppressor of normal
358 hematopoiesis¹⁰⁸. In both studies, inhibiting this niche remodeling led to enhanced chemotherapy
359 efficiency. These models may provide insight into how solid cancer DTCs may change the niches in
360 which they reside over time until they switch from a homeostatic pro-dormancy function to a
361 reactivation state. Given that the molecules derived from these niches may be more abundant than
362 the DTCs that lodged there, these soluble factors could serve as biomarkers that inform on niches
363 supportive of reactivation.

367 **FUTURE PROSPECTS**

368 Metastasis treatment has been limited to very few adjuvant therapies relying on primary tumor
369 data and aggressive targeting of metastatic lesions in stage IV patients. The latter is a necessity as
370 these patients have few treatment options. However, the knowledge surrounding tumor dormancy has
371 revealed that MRD in solid cancers could be targeted earlier, even if DTCs are not actively
372 proliferating¹⁰⁹. Additionally, the notion that dormant DTCs co-exist with growing lesions in stage IV
373 cancer has raised the possibility that these dormant DTCs may evade anti-proliferative treatments.
374 Thus, strategies that combine anti-proliferative therapies with drugs that kill dormant cells may
375 provide long-term benefit even in stage IV patients.

376 However, the above concepts need to be tested in practice. To accelerate our understanding
377 of the processes underlying cancer cell dormancy and consequently way to target it, we need to be

378 pairing experimental models with human data, for example by having research scientists team up with
379 clinicians to incorporate the measurement of endpoints related to dormancy in existing clinical trials.
380 Such efforts are emerging: for example, NR2F1, a dormancy marker identified in experimental
381 systems, has been used to stratify DTCs and determine that patients with NR2F1^{high} DTCs had longer
382 bone metastasis free periods than those with NR2F1^{low} DTCs⁵⁷. Importantly, a proliferation marker
383 such as Ki67 could not provide the same information, supporting that dormancy markers may provide
384 additional insights compared to determining the snapshot proliferative status of DTCs. Such efforts,
385 even if initiated in small patient populations, would be key for the validation of experimental biology in
386 patients.

387 The notion that dormant cancer cells activate autophagy^{18,89,90,110} has also led to a Phase II
388 clinical trial using autophagy inhibitors such as hydroxychloroquine and/or mTOR inhibitors in
389 combination to treat breast cancer patients and to prevent recurrence (Clinical trial identifier:
390 NCT03032406). An additional clinical trial is focused on screening for the presence of BM DTCs in
391 patients within 5 years of having completed breast cancer therapy (Clinical trial identifier:
392 NCT02732171). Patients with BM-DTCs will be offered the possibility to participate in other trials
393 targeting DTCs such as NCT03032406.

394 The concept of reprogramming malignant DTCs into dormancy⁴⁶ has also led to a clinical trial
395 testing this concept in disseminated prostate cancer that has not yet become detectable by imaging
396 (Clinical trial identifier: NCT03572387). This clinical trial repurposed 5-azacytidine and *all-trans*
397 retinoic acid (both FDA approved drugs) to treat prostate cancer patients after hormonal ablation.
398 Although these studies are ongoing they supports the notion that foundational science can be used to
399 develop innovative trials that can be executed despite potential cost issues²⁴. Understanding how
400 dormancy develops in patients may also help guide trials that were supposed to target micro-
401 metastatic disease but failed to show therapeutic value. Preclinical data have identified RANKL as a
402 tempting target with strong potential to prevent breast cancer bone metastasis^{111,112}. A phase 3 study
403 (D-CARE) combining a human blocking monoclonal antibody against the RANKL receptor (TNFSF11-
404 denosumab) with standard-of-care adjuvant or neoadjuvant systemic therapy and locoregional
405 treatments was developed. However, this treatment did not improve disease-related outcomes for
406 women with high-risk early breast cancer¹¹³. Reasons for this outcome may include the possibility of
407 dosing schedules leaving a window for dormant DTC populations to become reactivated. There is
408 also evidence from other studies that chemotherapy causes severe alterations of the bone marrow
409 microenvironment¹¹⁴ with clear signs of premature aging that could actually promote dormant DTC

410 reactivation. Unfortunately, the trial did not include intermediate endpoints to assess DTC or
411 circulating tumor cell numbers or phenotypes and thus to anticipate what may be happening to the
412 residual disease. Developing markers and methods to detect the DTCs that lead to MRD in such
413 trials, will enhance our understanding of how to target these residual cells based on their unique
414 biology.

415 The progress in on cancer dormancy research has expanded significantly and will likely yield
416 innovative biomedical results in the future. The findings discussed in this article highlight that the time
417 is ripe to start translating the knowledge gained from experimental models to the clinic.

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432 **Author Contributions**

433 E.R., A.R.N, V.M.S and J-A.A-G conceived the article, performed literature searches, integrated the
434 information wrote, discussed and edited the manuscript.

436 **Competing interests statements**

437 Dr. Julio Aguirre-Ghiso (a Co-author in this article) is a scientific Co-Founder of, Scientific Advisory
438 Board Member and equity owner in the private company, HiberCell LLC. In addition, Dr. Aguirre-
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440 Sinai spin-out company focused on the research and development of therapeutics that prevent or
441 delay the recurrence of cancer.

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772 **LEGENDS**

773 **Figure 1. Differences and commonalities between normal quiescent, senescent, differentiated**
774 **cells and dormant cancer cells.** Cell cycle arrest and survival programs are shared across all
775 programs. Although differentiation and senescence are typically irreversible or highly stable stages,
776 quiescence and dormancy are reversible. It would appear that normal quiescent and cancer dormant
777 cells interpret the same microenvironment factors in a similar manner leading to the activation of
778 survival and self-renewal programs. However, dormant DTCs also turn on diapause and pluripotency
779 programs and a UPR also observed in senescent cells. Normal quiescent, senescent and
780 differentiated cells are shown in green⁴⁴; dormant cancer cells are shown in blue²⁶. Circles: cytokines;
781 arrows: activated programs; arrows + cross: repressed programs.

782

783 **Figure 2. The different BM niches, cell types and cues regulating DTC and HSC dormancy.**
784 Both the osteoblastic and perivascular niches in the BM are involved in DTC dormancy by producing
785 a wide range of cues (cytokines, miRNA, extracellular-vesicles, cell-cell contact signalling, etc) that
786 drive dormancy. Additionally, dormant cancer cells downregulate antigen presentation and upregulate

787 immune-suppressive ligands in order to evade immune recognition. Over time and reciprocally,
788 cancer cells also remodel their surrounding microenvironment hypothetically feeding a proliferative
789 positive loop.

790
791 **Figure 3. Cues, receptors and cell types involved in DTC reactivation and pro-dormancy**
792 **niches.** In the BM, a pro-dormancy niche involves the action of several factors (such as LIF, TGFB2,
793 GAS6, BMPs; see Table I for more details) leading to the induction of both DTC and HSC
794 quiescence. In contrast, reactivation niches in the lung and liver awaken dormant DTCs, in a manner
795 that involves formation of NETs, extracellular matrix stiffness, TGFB1 and BMP inhibitors.

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	Factor	<i>In vitro</i> data	<i>In vivo</i> data	Human data	References
DORMANCY INDUCERS	CXCL12^{1,2,3}	Y	Y	Y	65
	VWF^{1,2}	Y	Y	N	23
	TGFβ-2^{1,2}	Y	Y	N	36
	BMP4/BMP7^{1,2}	Y	Y	N	43,48
	HYPOXIA^{1,2,3}	Y	Y	Y	33
	GAS6	Y	Y	N	115,116
	RA^{1,2}	Y	Y	N	46
	LIF^{1,2}	Y	Y	N	97
	TSP1^{1,2}	Y	Y	N	31
	WNTs^{1,2}	Y	Y	N	27,117
	DNA methylation Repressive chromatin state^{1,2,3}	Y	Y	Y	46,72
	Wnt5a^{1,2}	Y	Y	N	72
	Axolotl embryo¹	Y	N	N	86
	miR-126^{1,2,3}	Y	Y	Y	104
Jagged 1^{1,2}	Y	Y	N	118	
REACTIVATION INDUCERS	Coco^{1,2,3}	Y	Y	Y	48
	Inflammation/NETs^{1,2,3}	Y	Y	Y	32,75,76
	Stiff collagen^{1,2}	Y	Y	N	79
	Aging^{1,2,3}	Y	Y	Y	78,79,119
	VCAM1^{1,2?}	Y	Y	N	60
	Periostin^{1,2}	Y	Y	N	31
	TGFB1^{1,2}	Y	Y	N	36

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Table 1. Summary of dormancy and reactivation inducing factors. List of known cancer-cell- and microenvironment-derived regulators promoting dormancy or reactivation of dormant cancer cells in solid and liquid cancers. The type of data available for each factor are also indicated. These findings

806 are compiled from different cancer types, and thus these signaling pathways might not all be
807 implicated in dormancy of every type of cancer or microenvironments.

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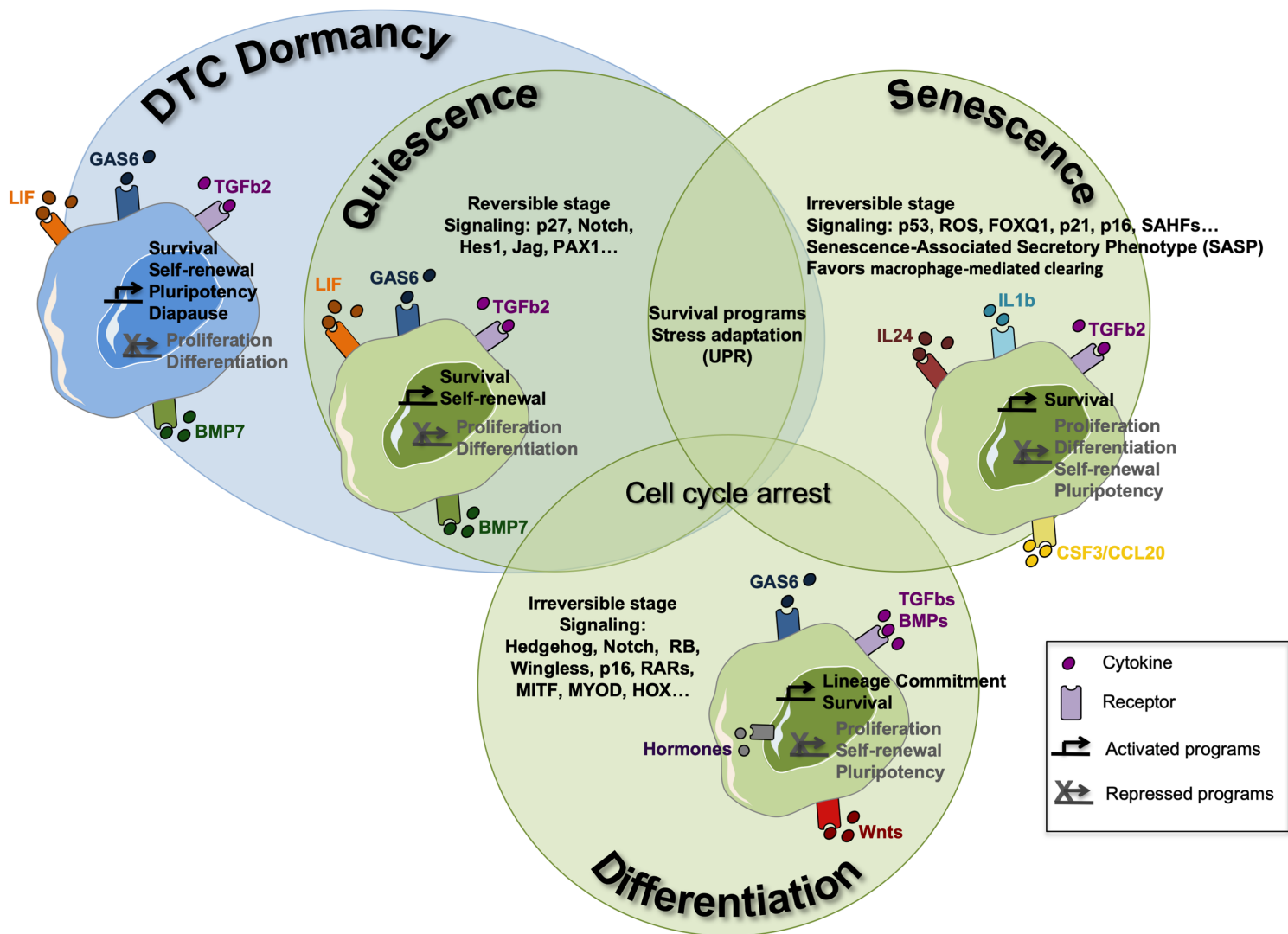


Figure 1. Differences and commonalities between normal quiescent, senescent and differentiated cells (all in green)⁴⁴ and dormant cancer cells (blue)²⁶.

OSTEOBLASTIC NICHE

PERIVASCULAR NICHE

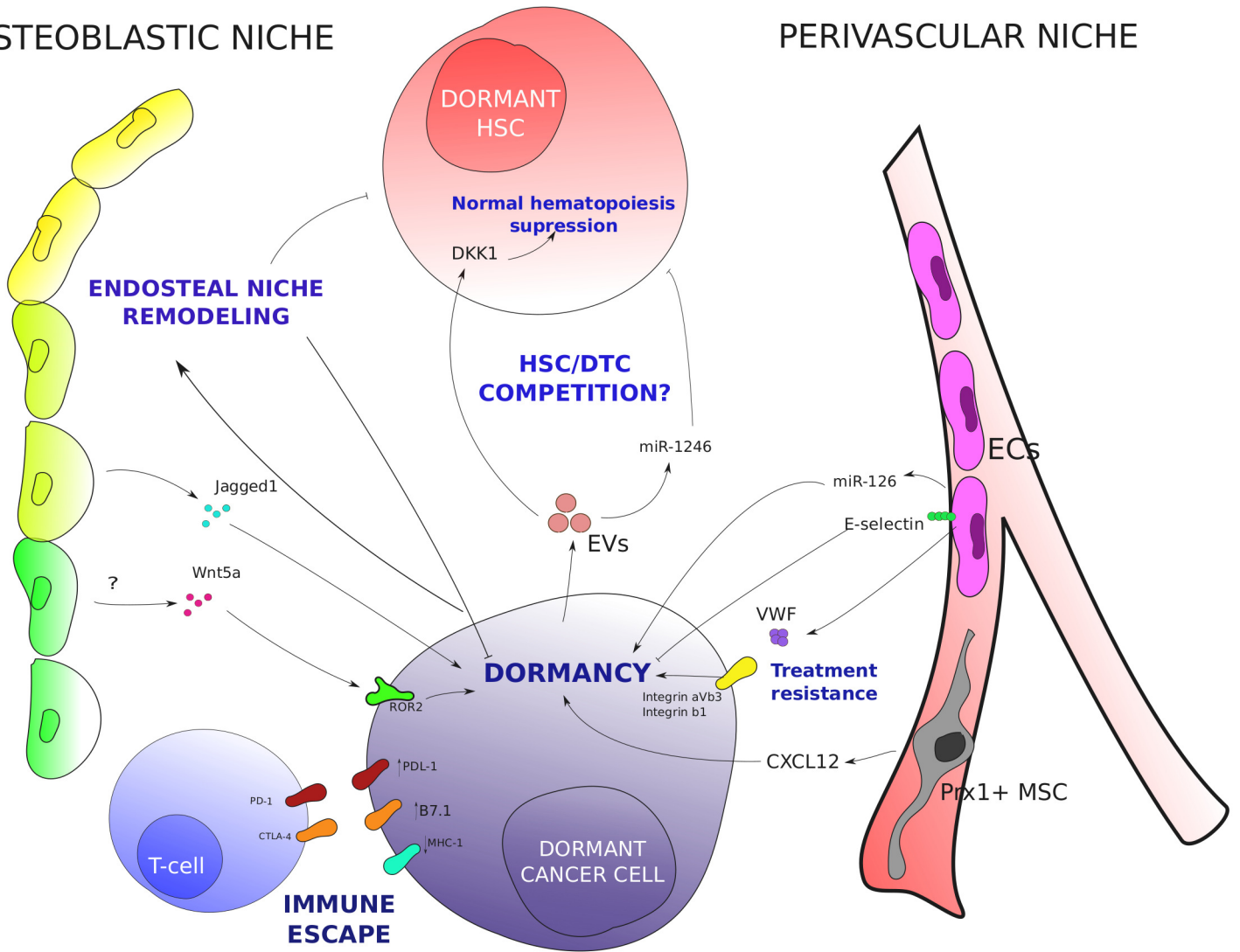


Figure 2. The different BM niches, cell types and cues regulating DTC and HSC dormancy

REACTIVATION MICROENVIRONMENT

BM PRO-DORMANCY NICHES

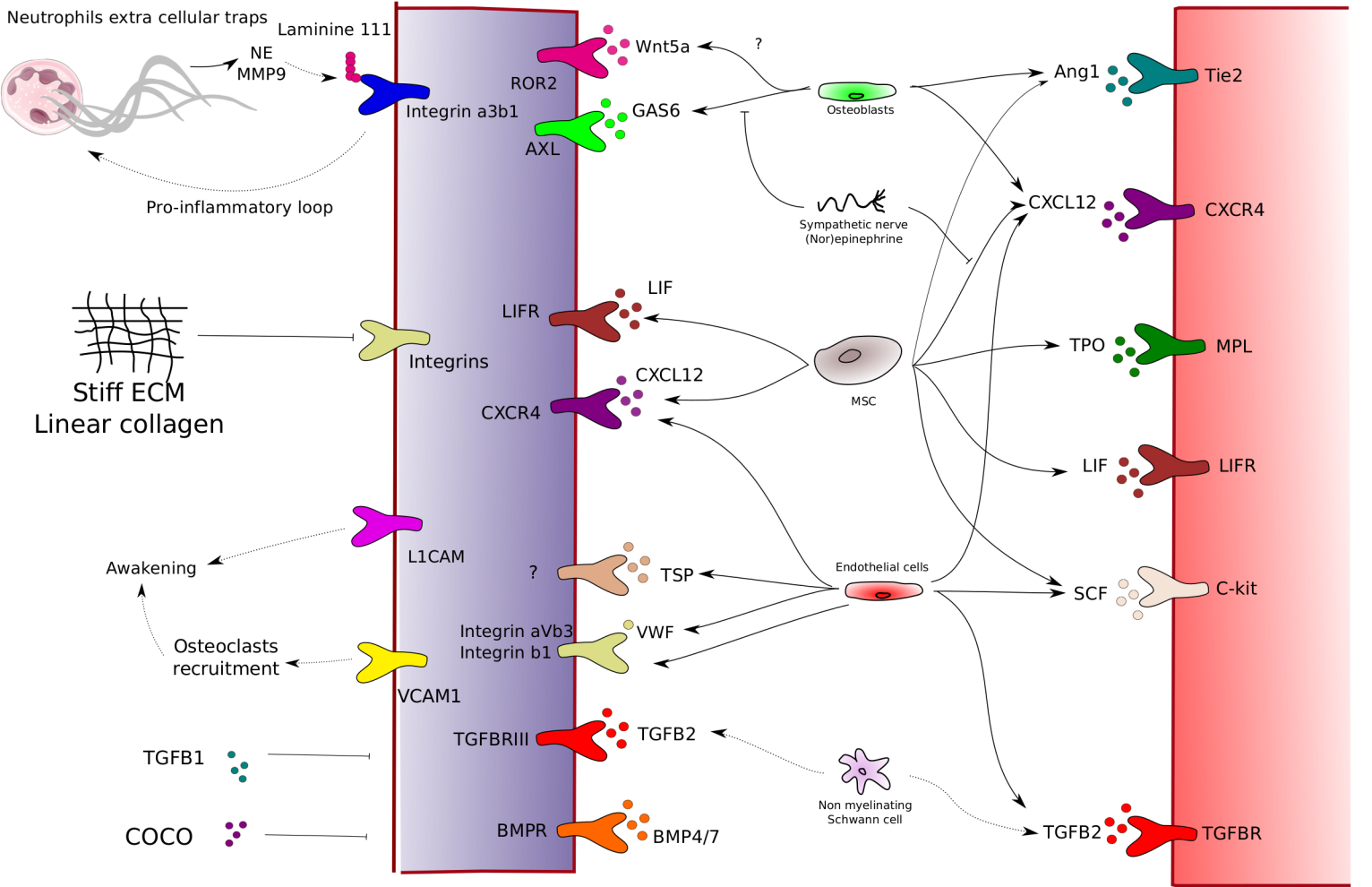


Figure 3. Cues, receptor and cell types in reactivation and pro-dormancy niches

