# Fluids and mechanics in tumor transit: shaping metastasis

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**Abstract**

Cancer metastasis is a dynamic succession of events that allows tumors to spread to distant sites within the body, ultimately reducing cancer patient survival. To colonize distant organs and thus disseminate over the organism, cancer cells and associated factors exploit several bodily fluids, which provide a natural transport route. The flow mechanics of the blood and lymphatic circulatory systems can be co-opted for efficient cancer cell transit, extravasation and metastatic seeding. Flow rates, vessel size, and shear stress can all impact on the survival of cancer cells in the circulation and control organotropic seeding patterns. Thus, in addition to using these fluids as means to travel throughout the body, cancer cells exploit the underlying forces to successfully seed distant metastasis. The contribution of bodily fluids, and their mechanics, raises interesting questions about the biology of the metastatic cascade; its understanding may therefore provide a new avenue for targeting cancer cells in transit.



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## Introduction

"*Panta Rhei*", or literally "*Everything flows*": this concept, often attributed to the presocratic greek philosopher *Heraclitus* (500 B.C, but also to *Simplicius*), was an attempt to explain the ever-changing nature of life. Recent work has shown that this might also apply to tumour metastasis. Tumour metastasis is a complex multi-step process where malignant tumours shed invasive metastatic cells that need to overcome many obstacles (ex: evading immune surveillance, etc..) for successful metastatic outgrowth[1]. However, in addition to these multiple molecular pathways, a plethora of studies conducted in the past two decades strongly suggests that mechanical forces are also responsible for tumour progression and response to classical therapies[2–4]. Among these forces, fluid-based mechanics progressively enter the scene. Indeed, on their way to metastasis, tumour cells and secreted factors use and exploit three main bodily fluids **[G]***: blood, lymph and interstitial fluids[5–7] **[G]** (Fig. 1). Circulating tumour cells (CTCs) **[G]** and their associated material (soluble factors **[G]** and extracellular vesicles, EVs **[G]**) can directly travel through the hematogenous system[1,8] or sequentially use both the lymphatic and blood vasculature to colonize distant organs[9–11] (Fig.1A,B). An early seminal study coined the "hemodynamic theory" showed that arterial blood flow in certain organs can be positively correlated to metastasis frequency[12] supporting an actual link between flow mechanics and the secondary site of metastasis.

When transported in fluids, CTCs are subjected to and exploit various mechanical forces, which can impact their fate in many different ways. For instance, high shear forces exerted on CTCs can induce mechanical stress leading to cell fragmentation and death[13], while intermediate shear forces have been shown to favor CTC arrest and extravasation[14]. Therefore, a greater understanding of the mechanical forces encountered by CTCs and tumour-associated material in fluids is crucial to understand the metastatic cascade and delineate vulnerable CTC states for therapeutic intervention.

In this review, we describe how circulating tumour material (cells and associated factors) use bodily fluids and their underlying forces and stresses as natural means to escape primary tumours, travel throughout the body, prime pre-metastatic niches **[G]** and successfully seed distant metastasis. We briefly review key flow-related



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aspects of tumour growth and invasion that have received significant attention[2,7] and discuss how these modes of flow now become essential transport means for tumour-derived material[15,16]. We detail the impact of flow types, patterns, components and flow-associated forces during hematogenous dissemination, with a focus on the last steps of tumor metastasis. We also detail the existing palette of *in vitro* and *in vivo* models as well as multi-scale imaging and biophysical tools that are available to the tumour metastasis research community for interrogating fluid mechanics and intravascular behaviour of CTCs during tumour metastasis. The contribution of fluids, and its mechanics, raises interesting questions about the fate and metastatic potential of CTCs and thus the biology of the metastatic cascade, and opens up an important area often overlooked in cancer research that may be susceptible to future targeting.

Due to their different composition and characteristics, blood, lymph and interstitial fluids can be described by different biophysical attributes that allow us to estimate the mechanical stress imposed on CTCs and other blood components[17,18]. In Table 1 (and Fig.1C), we outline some key features of fluids likely to transport tumour material and describe the flow rates often experienced or exploited by CTCs and/or tumour-secreted material during transit within each of these interlinked compartments. For instance, lymphatic flow is mostly laminar **[G]**, pulsatile with a low amplitude, is mainly driven by viscosity and displays low velocities[19,20]. By contrast, blood has a much higher density of circulating objects (blood cells and other factors) and is associated with higher flow velocities due to cardiac pumping. In addition, blood flow can be pulsatile with a high amplitude and turbulent in arteries, while it is mostly laminar in veins[21,22]. Furthermore, biophysical cues differ according to flow type and vessel type (Table 1), and organ (Fig.1C). Overall, CTCs and tumour-derived material traveling in the circulation are exposed to shear rates ranging from ~$10s^{-1}$ in the lymph[19], to ~$1000s^{-1}$ in large arteries[22,21]. These two main fluids are also responsible for the interstitial flow that is characteristic of tumours[2]. Interstitial fluid is generated by a high capillary blood pressure as well as cellular pressure in the solid tumour and is drained throughout the lymphatic system and its primary valves[23] where the pressure is lower. This directional displacement has been described in a model[24] based on Darcy's law[25] **[G]** and is mainly linked to the pressure difference, the surface area and the hydraulic conductance **[G]** between these networks.



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Interestingly, interstitial fluid pressure has important consequences for the dissemination of tumor cells and tumor associated material[7], but also for drug delivery into the tumour[2] (Box 1).

## Fluid mechanics in primary tumors

Solid tumours form a complex tissue containing cancer cells, stromal cells, extracellular matrix (ECM) as well as blood and lymphatic vessels. Tumour growth relies on angiogenesis and lymphangiogenesis[26,27] **[G]**. While the first provides oxygen and necessary nutrients through new blood vessels formation, the second removes excessive fluids from tissues but can also drain tumour cells and tumor-secreted factors (Fig. 1). In addition, both fluid systems allow the transit of immune cells in and out of distinct tissue, and the lymphatic circulation orchestrates local immune surveillance under the control of neighbouring lymph nodes[15,28].

Darcy's model explains how pressure differences drive diffusion and convection of fluids from the blood towards the draining lymphatic vascular system[7] (see Box 1 and Table 1). In primary tumours, this pressure difference is further enhanced by the leakiness of the non-mature tumor-associated blood vasculature[29,30]. In addition, rapid tumor growth creates solid stress **[G]** that exposes the tumor-associated vasculature to further vascular squeezing through compression and tension[31–33] (Box 1) thereby generating high interstitial fluid pressure (IFP) in the tumor tissue. It has been shown in the case of murine pancreatic ductal adenocarcinoma that intratumoral IFP can increase to levels more than 9-fold compared to IFP measured in matched healthy tissue[34]. While high IFP is measured in the tumour core[34,35], IFP levels drop at the tumor periphery and thereby establishes interstitial flow through the peritumoral stroma into the lymphatic vessels[7,36] (Fig. 1 and Box 1B-C). The interstitial flow results from both convection and diffusion flow towards the periphery[37] with velocity values that approximate 1-4μm/s[38–40]. In parallel, solid stress **[G]** squeezes blood and lymphatic vessels[41], and thereby hinders oxygenation and homeostasis of the solid tumour. In doing so, tumor cells tend to release more angiogenic factors[23], that lead to an aberrant and hyperpermeable blood circuit (Box 1A, low velocity, highly heterogeneous) and thus further contribute to the malignant behaviour of tumour cells.

Several studies showed that tumor interstitial fluids **[G]** promote the migration of tumor cells. Indeed, convective interstitial flow **[G]** can, for example, increase



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glioblastoma cell invasion[42] and promote amoeboid phenotype motility of breast cancer cells [43] towards the lymphatic drainage where tumor cells have been shown to escape from the primary tumour with the help of the immune system[44] (Box 1D). Interstitial flow can also influence stromal cells by promoting the differentiation of macrophages into polarized populations that were shown to increase directional cancer cell migration[44]. Macrophages were also shown to assist tumor cells (melanoma, breast and protaste human lines) during intravasation[45,46], but whether this can be influenced by interstitial flow is not known. Furthermore, cancer cells can move against the convective flow using substrates, such as collagen I fibers, to adopt a mesenchymal motility and phenotype[43]. An elaborate *in vitro* study also showed that interstitial flow can synergize with luminal vessel flow to enhance tumour cell intravasation within lymphatic vessels[47]. However, whether this also happens *in vivo* remains unknown.

By influencing the direction of tumour cell migration, interstitial flow can guide tumour cells and associated factors to the vicinity of lymphatic or blood vessels. Indeed, it is tempting to suggest that such convection forces, in combination with blood and lymphatic circulations, displace tumor-derived material such as tumour-derived cells, thus favoring their directed dissemination, but also soluble factors or extracellular vesicles (EVs, please see below for more detail) towards the vascular systems or towards the ECM at the periphery (Box 1A-D), while also limiting therapeutic distribution in solid tumours[23] (Box 1E).

**Two exit routes to escape**

Recent evidence obtained in patients with breast[48] and colorectal carcinoma[49] suggests that tumour dissemination is an event that can take place in early lesions rather than in more developed primary tumours. Although this needs to be confirmed in other cancer, such feature has been confirmed in mouse models of pancreatic[50] and breast cancer[51–53] and raises the question of early dissemination mechanisms. While early lesions are less likely to be highly vascularized, whether fluid mechanics impact intravasation in early *versus* advanced primary tumours remains to be determined. Whereas some cancer types directly disseminate through the blood vasculature, others spread via the lymphatic circulation (Fig. 1A). Hematogenous dissemination of CTCs follows successful intravasation of individual (as in the case of spontaneous murine mammary tumors[45,54]) or cohorts of invasive carcinoma cells



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(that are rare in comparison to single CTCs in breast and prostate cancer patients[55]) into the vasculature of neighbouring normal tissue or the vasculature formed within the tumours[55] (see Fig. 1B). How flow mechanics impact intravasation of single versus clusters of tumour cells, and whether this impacts the flow type they favour for initial dissemination, remains to be determined, and microfluidic approaches (Table 2 and Supplementary Information S1) could be instrumental to answering these questions. Cancer spreading to regional lymph nodes often precedes systemic disease and is the most reliable factor for predicting survival in patients with carcinomas[56]. Lymph node metastasis defines poor outcome in many cancers such as lung, prostate, breast and colorectal carcinoma as it correlates with spreading to vital organs[57]. Although it is not causing death of patients, lymph node metastasis is thus reliably used for staging and predicting cancer progression. Interestingly, tumour cells that had metastasized to lymph nodes via the lymphatic circulation can transfer to the blood circulation that perfuses the lymphatic organs (Fig. 1A,B). Because tumour spreading to the lymph nodes often correlates with reduced survival[58], lymph node metastases are thought to be intermediate steps for life-threatening distant metastases, although their removal is not beneficial for melanoma patients[59]. Demonstration for such intermediate transit has also been provided in mouse models of melanoma, mammary, colorectal and squamous cell carcinoma[9,11]. Careful tracing of the fate of tumour cells revealed that they initially colonize the cortex of lymph nodes upon draining from the lymphatic circulating through the subcapsular sinus. There, tumour cells exploit endothelial venules to exit from the lymph node into the blood vasculature before seeding secondary metastases in the lungs, which can also form directly through hematogenous dissemination[11]. Interestingly, when studying the evolutionary history of metastases in the context of human colorectal cancer, researchers found two distinct lineage relationships between lymphatic and distant metastases that are intricately linked to fluid routes[10]. For example, in human colorectal cancer patients phylogenetic analysis of matched primary tumors and metastases showed that while 65% of patients displayed distinct origins for lymphatic and distant metastases, 35% of distant tumors shared a common metastatic ancestor with lymph node metastases[10]. Whether fluid biomechanics play a role in the initial choice of the fluid route is not known. However, since flow velocities and shear stress **[G]** are lower in lymphatic than in blood vessels (in rats)[19] (Table 1 and Fig. 2),



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lymphatic dissemination might be initially less deleterious to CTCs than dissemination through the blood.

**Dissemination of tumor-derived factors**

In addition to representing fast escape routes for tumour cells, lymphatic and blood vessels allow the dissemination of various tumour-secreted factors in the organism[15,16,60–64]. Tumour-secreted factors can include cytokines, chemokines, growth factors, matrix metalloproteinases (MMPs), circulating DNA, antigens, or extracellular vesicles (EVs). How these factors enter the circulation has not been formally demonstrated, but they are likely to be transported by interstitial fluids and, in contrast to tumour cells, unlikely to be transported against the flow (Fig.1B). Recent development in tracking of EVs *in vivo* could be instrumental to identify these early dissemination routes[65]. Chemokines have been shown to diffuse as gradients in the interstitial fluids and to guide dendritic cells toward lymphatic vessels in mice[66]. Here, similar mechanisms could favour the dissemination of tumour-secreted factors. Tumour-secreted factors are found in both blood and lymphatic circulation, where they constitute novel targets for early cancer diagnosis or longitudinal monitoring of anti-cancer treatment response using liquid biopsies, in addition to CTCs[67,68]. Given their short half-life in the circulation (around 2 minutes for EVs[69,70] injected intravenously in mice and less than 115 minutes for circulating DNA[71] in human) but their high abundance in body fluids, it is likely that they are permanently released by tumors. Both physics and biology provide reasons for these short half-lifes. The clearance of EVs from the circulation depends on myeloid cells, in particular macrophages, as shown in mice and zebrafish[72,73] and neutrophils in mice[74], which are present in blood vessels and on endothelial cells. The small size of EVs favors their margination **[G]** close to the endothelium walls, similarly to synthetic nanoparticles[75,76], therefore enhancing the probability of being ingested by phagocytic cells, as recently observed in zebrafish embryos[73]. The size of EVs, which range from 35nm for the recently described exomeres **[G]**[77] and up to a few microns for oncosomes **[G]**[78], might also affect their repartition in the blood flow, and ultimately their organ distribution, as shown for gold nanoparticles in rats[79]. One could also speculate that EVs' surface repertoire changes whilst in the bloodstream compared to extravascular EVs, leading to a change in their interaction dynamics with the microenvironment whilst in flow. In addition, EVs that are not rapidly taken



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up might be subjected to the high shear characteristic of arteries, and thus potentially be destroyed as has been shown for breast, ovarian lung and leukemic CTCs in a microfluidics system[80]. In mice, tumor-derived EVs are also rapidly transported in lymphatics[81], where they end up in metastasis-free lymph nodes and can be internalized by resident subcapsular sinus macrophages[82,83]. Similar observations were made for the dispersion of tumour antigens[84] and circulating tumour DNA[85].

Tumour-secreted factors can arrest in various organs free of metastasis where they can create pre-metastatic niches by locally altering the microenvironment prior to the arrival of tumour cells[60,62,64,86]. For instance, uptake of pancreatic tumor EVs by kupfer cells in mice liver induces TGFβ secretion, which leads to changes in the extracellular matrix thereby promoting the recruitment of metastasis supporting bone marrow derived macrophages and granulocytes[54]. The complex molecular and cellular composition of pre-metastatic niches facilitates subsequent efficient colonization and survival of pioneer metastatic cells[87]. Remarkably, depending on their tumor origin, EVs can target specific cells and organs in mice [62,64]. For example, EVs from human breast cancer cells that metastasize primarily to the lung are mostly taken up in lung fibroblasts after intravenous injection in mice[56]. This organ-specificity relies on the repertoire of integrins present at the surface of EVs[56]. However, the identity of the integrin ligands involved in this process is not known, as well as the organ distribution of these ligands. More generally, how soluble and vesicular tumor factors cross the endothelium to escape the circulation in specific organs is not clear. It could be linked to the capacity of tumor EVs to induce endothelial permeability[88–90]. For example, melanoma-shed EVs promote vascular leakiness and educate pro-metastatic bone-marrow progenitor cells[64] and multiple myeloid cells in mice. To date, the impact of flow forces on circulating tumour factors has been very poorly described. Recent observations show, however, that EVs circulate with reduced velocities in the proximity of the vessel walls, following the Hagen-Poiseuille equation **[G]** which states a quadratic flow velocity profile maximal at the centre and minimal close to the vessel walls, and tend to arrest in regions of low blood flow in zebrafish[73,91]. Since these regions are also the sites of efficient extravasation of CTCs[14], it is tempting to speculate that the seeding of premetastatic niches by EVs on specific organs and their subsequent colonization by CTCs happens in regions which are sharing similar shear flow profiles. This can now be investigated using



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novel animal models allowing the tracking of EVs in the circulation *in vivo*, such as zebrafish embryos[73,91] (Table 2 and Supplementary Information S1), which opens new avenues for understanding the priming of metastatic niches at high spatio-temporal resolution.

## The intravascular journey of CTCs

CTCs use various means to enter into both the lymphatic and vascular circulation. Their dissemination is tightly linked to fluid routes[92] and arterial flow profiles[12] (Fig. 1A and 2, Table 1). The dissemination of CTCs can be analysed using a still-growing platform of animal models and experimental approaches (Table 2, Supplementary Information S1 and quantitative methods in Table 3) and recent developments in 3D printing technologies now allow to design human-like vessels at will and are likely to provide unprecedented insights when applied to flowing properties of CTCs (Table 2).

### *Shear stress depends on flow types and vascular regions*

Invasive tumour cells enter the lymphatic and venous blood vessels, with low velocity and laminar flows and where fluid dynamics is predominantly driven by viscosity[93] **[G]** in opposition to the flow in arteria that is mostly driven by inertia **[G]** [94,95] (Table 1). Within the proximity of the primary tumour, where the vasculature is capillary-based, CTCs (as erythrocytes[18]) are exposed to moderate stress due to low flow velocity. The intravascular flow in blood capillaries can be modeled not only using classical fluid dynamics (Table 1) but also needs to take into account the visco-elastic behavior **[G]** of the endothelial vascular barrier. Thus, this introduces the fluid-structure behavior **[G]** with blood vessels inflating when the blood pressure increases due to heart contraction. The endothelium, thus stores energy, that is released by deflating and helps the flow pressure propagation, lowering the cardiac work[93]. Flow mechanics slightly differ between animal models and between organs (Fig.1C). However, we believe that some animal models, such as the zebrafish embryo, allow a very reliable comparison in terms of capillary-like hemodynamics. Capillary diameter, blood velocity, Reynolds number and shear stress are very comparable among all models. As an example, hemodynamic features of the zebrafish embryo ressembles ones from the human brain which demonstrates that the zebrafish embryo can be used as a model for tracking tumor metastasis mechanisms, when



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properly validated in a mammalian model, as we have published in the past[96]. In addition, our observations (high-resolution electron microscopy) show that vascular remodeling is very similar between the mouse and the zebrafish model. This suggests that similar mechanisms are used, independently of vascular basement membranes and ECM cross-linking, and could fully rely on the similar hemodynamic profiles observed".

During their journey in the blood vascular system, some of the CTCs will reach the arterial circulation where flow shear stress and velocities are high (Fig 2, Table 1). Here, they might undergo deformation due to high shear, causing fragmentation and death. In addition, CTCs are known to circulate, in minor proportion, as clusters in mice and human [55,97]. It has been shown that the trajectories of these clusters in the bloodstream depend on their size and shape. Compact clusters (triangular or square shaped) will flow in closer proximity to the endothelial barrier than linear clusters[98,99]. Such behavior might increase their probability of engaging in adhesion with the endothelium, which can be further potentiated by the increased density of adhesion proteins[100].

In regions where the vasculature undergoes ramification **[G]** , flow remains pulsatile with lower velocities and is predominantly driven by the viscous contribution (Table 1), as defined by fluid-structure modelling[14,93] **[G]**. Thus, CTCs in flow are less exposed to shear stresses. Shear stress increases during intravascular arrest (see next section), that follows either active adhesion to the endothelium or by mechanical trapping as observed in mice brain or lungs and in rat livers[13,101,102]. During their journey in the circulation, tumour cells sense shear stress[103] of the flow that carries them and need to adapt their behaviour to arrest and extravasate. Mathematical models that mimic blood flow in capillaries and simulate erythrocytes have been developed[104] and are likely to provide unprecedented insights into intravascular behavior of CTCs. Recent improvement of computer power now allows to simulate blood flow of human pulmonary capillaries[105], where complex erythrocyte deformation is integrated[106]. Such tunable models can now be applied to flowing tumour cells and show that CTCs need to adapt the stiffness of their cytoskeleton in a very specific way to reach full adhesion on the endothelial wall[107]. Similarly, margination properties **[G]** of single versus clusters of CTCs can now be studied [108]. These models ranges from the single CTC in absence of erythrocytes[107], complex



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flows populated with erythrocytes subjecting CTCs to margination[108,109], to thromboembolism of CTCs[110,111].

CTCs are thus subjected to various hemodynamical forces while in transit, which significantly depend on flow types, vascular regions and organ. Further work is needed to determine whether the impact of flow profiles and vascular architecture on CTCs' behaviour differs between metastatic organs (lungs, brain, liver, bone marrow see Fig.1C). However, CTCs have evolved mechanisms to resist and survive these intravascular stresses.

***Impact of shear forces on CTCs***

When flowing as single objects, CTCs are likely to interact with each other within the bloodstream (see next section) or collide with blood components and the vasculature walls. Metastatic tumor cells have been shown to disseminate rather efficiently from the primary lesion[112]. Interestingly, when injecting tumor cells in the afferent vessel of metastatic organs (ex: melanoma cells in the portal vein for liver[113] or in the vena cava for the lung analysis[114]), less than 4% of injected cells form efficiently micrometastatic foci, although 80% of the cells are thought to stably arrest at 1 day post-injection[113,114]. In the context of murine brain metastasis, only 40 to 60% of the arrested cells do sustain the ripping forces of the blood flow and extravasate[102]. This suggests that inefficiency of the first metastatic steps originates from intravascular pre-extravasation event. Further deleterious effects to metastasis are driven by the immune system[115], or by microenvironment niches that restrain growth or induce dormancy[113,114,116]. Recent work in mouse models of breast cancer metastasis show that some CTCs have developed organ-specific survival strategies (for example, oxidative stress and counteracting antioxidant programs in the case of lung metastasis) to successfully seed secondary sites[117]. CTCs suffer from shear and collisions that can induce cell cycle arrest (demonstrated for a shear of 12 Dyn/cm$^2$ [118]) (Fig.2) and physical damage and necrosis[119] (caused by shear values of ≈ 6 dyn/cm$^2$ [120] and gradually increasing with laminar shear stress[121]). Moreover, programmed cell death leading to apoptosis, is favored by shear stress *in vitro* with shear values of only 2 dyn/cm$^2$ [122]. Such low shear stress reduces the metastatic efficiency[123]. Interestingly, oscillatory shear stress of ≈ 4 dyn/cm$^2$ had no effect on death of human tumor cells suggesting that the type (and duration) of flow is equally important[121]. Of note, this remains largely underexplored and seems to be highly



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dependent on the cell type. Intriguingly, some CTCs can survive to high shear values (60 dyn/cm$^2$, for hours)[80]; whether they develop increased metastatic capacities remain to be explored.

Interestingly, comparison of human breast cancer primary tumor cells and CTCs suggests that flow-mediated DNA damage and subsequent mutations in surviving CTCs is linked to increased resistance to chemotherapy and metastatic seeding[124]. CTCs of various origins that are exposed to short but intense and disruptive shear stress pulses *in vitro* can repair their plasma membrane[125] through a calcium-dependent vesicle fusion mechanism that could be similar to what has been described in starfish oocyte[126]. Intravascular survival of CTCs also relies on nuclear resistance to stress that can be mediated by lamin A and C[120,127] or that could be mediated by the small GTPase Rac1 under the control of its STEF/TIAM2 guanine nucleotide exchange factor .

Shear forces can even initiate signalling pathways that drive resistance mechanisms. For example, CTCs from breast, prostate and lung cancer patients show increased expression of β-globin, which protects them from intracellular accumulation of reactive oxygen species (ROS) induced by shear stress[129]. Similarly, flow-dependent activation of mitochondrial mnSOD decreases ROS level[130]. In addition, reversible metabolic adaptations, such as activation of the folate pathway, allow CTCs to withstand oxidative stress readily adjust to new environments and survive in transit, as described in the context of human melanoma xenografts[131]. CTCs also endure anoikis[132] that commonly triggers apoptosis in tumour cells by the loss of cell attachment to ECM components. While this aspect has been mostly studied using cells in suspension, and thus the contribution of flow forces remains to be fully demonstrated, several molecular mechanisms have been identified. For example, cell adhesion proteins such as integrins[133] efficiently block anoïkis (as demonstrated on human samples[134]). Integrin signalling can suppress anoïkis by internalizing ECM fragments (and their own activated integrins subunits) into endosomal compartment [135]. In addition, anoïkis can be blocked upstream of integrin signaling and independently from ECM binding by ZNF304 (Zinc-finger transcription factor)[136]. Similarly, using cells in suspension and metastatic assays in mice in the context of intestine[137] and hepatocellular carcinoma[138], expression of the TrkB receptor[137] or the



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binding of Midkine to its receptor[138] activate PI3 kinase to block anoïkis and thereby increase metastatic spreading.

Shear forces also activate other transcriptional programs in CTCs. For instance, using microfluidic approaches (Table 2 and Supplementary Information S1), lymphatic-like (but not vascular-like) fluid shear stress promotes cell migration and motility in a YAP1-dependent manner [139]. Such shear also supports cell division in a TAZ-dependent manner [140] suggesting that CTCs can orchestrate various signalling pathways in response to shear forces *in vitro*. Whether CTCs behave equally *in vivo* remains to be determined.

Metastatic progression is often linked to epithelial-to-mesenchymal transition (EMT) at the primary tumor[141] and mesenchymal-to-epithelial transition (MET) upon arrival at the secondary site[142]. Interestingly, shear stress has been shown to trigger EMT in the context of several cancers *in vitro*[143,144]. However, CTCs tend to be very plastic and human CTCs from breast cancer, for instance, express both epithelial and mesenchymal markers[55,145]. Indeed, CTCs from breast or pancreatic cancers switch readily between epithelial and mesenchymal states, further favouring their survival as well as their invasive and metastatic potential in a diverse range of environments[146–149]. How blood flow influences MET/EMT programs, tumour cell plasticity and metastatic potential remains to be elucidated. Recently, shear stress-induced EMT was shown to promote stemness with high tumor-initiating potential[150]. This subpopulation of cells are characterized by reduced F-actin and stiffness as well as delayed adhesion, which altogether seem to promote cell survival[151].

CTCs are not equipped with the strategies that allow white[125,152] and red blood cells[153,154], for example, to resist to detrimental shear forces and are thus unlikely to successfully grow a metastatic colony. In order to survive, CTCs have developed means to interact with blood components or to exploit existing shear forces (Fig. 2). Clustering of CTCs, which can occur at intravasation[155,156], in transit[55,157] or at arrest sites[158], increases their resistance to detrimental shear forces as well as to assaults by the immune system[55], e.g. Natural Killer cells[159] (Fig.2). CTC clusters can be polyclonal with many cells only undergoing partial EMT while retaining some epithelial features[145], including cell-cell adhesion mechanisms[155]. Interestingly, members of the cadherin superfamily as well as plakoglobin were found to be expressed in CTC clusters from mice and human breast cancers[155,156,55] and thereby allow stable cell-cell adhesion while maintaining the CTCs' proliferative potential.



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CD44 was also shown to mediate homophilic interaction between CTCs in breast cancer patients[157]. In addition to mediating cell-cell adhesion in CTC clusters that is instrumental to their stemness properties and resistance to shear forces and anoikis, CD44 is a cancer stem cell marker[160] and is characteristic of tumour-initiating potential[161].

In addition to providing pro-survival properties, mathematical modeling suggests that travelling in clusters increases the drag force and decreases the speed at which these clusters were circulating, thereby favouring interactions with endothelial cells and the arrest of CTCs[98,99]. Clusters are more likely to physically lodge in microvessels at distant sites before seeding metastatic colonies as speculated from mice studies[55]. Of note, this metastable **[G]** arrest has been described as independent from the stable adhesion, mediated by integrins, in the context of metastasis in rat liver[101] (Fig. 2). Since mechanical constraints imposed by vessel architecture and size contribute to the arrest of CTCs[102], clusters of CTCs should be rapidly trapped and cleared from the circulation before reaching distant organs. Indeed, the half-life of mammary carcinoma clusters in circulation is reduced compared to single CTCs in mice[55]. Surprisingly however, clusters of CTCs are also capable of squeezing through capillary-sized vessels in microfluidics devices *in vitro* or in zebrafish *in vivo* [162] (Fig. 2) and resist to various blood-borne attacks from immune cells[132]. Clusters of CTCs have been shown to be able to reduce their hydrodynamic resistance by forming single-file structures that rely on intercellular adhesive interactions[162]. More work is needed to better understand whether the increased metastatic potency of CTC clusters can be explained by a higher propensity to arrest and to extravasate.

In addition to circulating as clusters, CTCs can directly interact with other cell types, such as cancer-associated fibroblasts (CAFs[163]) when in transit. These cells are derived from the stroma of the primary tumor and can accompany CTCs to their secondary destination[164–166], and these interactions between CTCs and stromal cells are likely to modify the intravascular behavior of CTCs and favour metastasis[138].

When circulating, CTCs are also no longer protected by the primary tumor microenvironment [167], and are more exposed to the immune system (Fig. 2), including circulating T-Lymphocytes and Natural Killer cells[168]. Here, CTCs are able to interact with and co-opt other cell types in the circulation to take advantage,



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escape the immune system and favour metastatic onset. Studies in mice show that CTCs can also be found in clusters with neutrophils in the bloodstream[169,170], and exploit them to bind to and arrest on vessel endothelial walls while resisting shear stress[169,171] (Fig.2). These heterotypic interactions between CTCs, neutrophils and the endothelium are mediated by a repertoire of cell adhesion proteins involving integrins, cadherins and cell surface glycoproteins, at least *in vitro*[143] and may provide another 'druggable' target axis to expose CTC vulnerability.

Furthermore, CTCs recruit and activate platelets[172], the latter step being also dependent on blood flow[173]. Interestingly, when interaction between CTCs and platelets is hampered during thrombocytopenia **[G]**, this correlates with reduced metastatic potency[174]. Platelets are thought to behave as a shield for CTCs against mechanical stress and immunosuppressive components[175,176]. They further allow the binding of tumour cells to the endothelial wall, through their natural ability to link PSGL1 (or PECAM) on endothelial cells[177,178]. Moreover, platelets can protect CTCs against anoikis in a YAP-dependent manner *in vitro* and promote metastasis in mice[179]. In addition to various pro-metastatic properties, platelets and specific platelets receptors were also shown to mediate anti-metastatic effects in murine models [180,181] questioning their exact contribution to metastatic onset or indicating a timing- or context-dependent role of platelets in the metastatic cascade. Although platelets' contribution to the metastatic potential of CTCs is unquestionable, more work is needed to clarify how platelets behave under shear conditions with respect to CTCs and CTC survival.

Recent development in single-cell molecular analysis now allow to capture and highlight strong intracellular heterogeneity within population of CTCs[182]. While such heterogeneity is caused by genetic instability or cellular plasticity, it is very likely that individual or cluster of CTCs differ between tumor types, patients and thus respond very differently to fluid flows, as it is the case with treatment resistance. Many pharmaceutical agents that target blood flow and blood flow mechanics have been approved for clinical use, such as the treatment of cardiovascular diseases. Recent data suggest that drugs, such as anticoagulants, beta blockers or ACE inhibitors may also have an impact on cancer metastasis. As discussed before, platelets have long been implicated to promote cancer metastasis[183]. For example, the anticoagulant hirudin was shown to profoundly reduce CTC numbers and metastasis in the 4T1 mouse model of breast cancer, potentially by disrupting the interaction between



*[G] : see Glossary, page 26

platelets and cancer cells[184]. Similarly, local pro-coagulative environments have been detected within the blood vessel lumen at primary and secondary sites where they may facilitate intra- and extravasation, and heparins targeting these environments were shown to reduce metastasis in pre-clinical cancer models[185,186]. Chronic stress induces beta-adrenergic signalling, which increases heart rate and changes in blood pressure. Stress-induced beta-adrenergic signalling has also been shown to promote metastasis while increasing density of blood and lymphatic vessels in the primary tumour, and elevating lymph flow rate in draining collectors, which all could be targeted using the beta-blocker propranolol[187,188]. Recent data demonstrate that beta-adrenergic signalling increases cancer cell stiffness during cell invasion[189] and it would therefore be interesting to see whether beta-adrenergic signalling can also protect CTCs from deformation and assault while traveling through the circulation. Interestingly, it was recently shown that short-term intervention using a combination of the beta-blocker propranolol and the COX2 inhibitor etodolac during the peri-operative period in breast cancer patients was sufficient to reduce pro-invasive and pro-inflammatory gene signatures in the tumour tissue[190]. Whether this transient intervention at a critical period, when patients are subjected to high levels of stress, can also affect CTC abundance and metastasis in the long term should be assessed in future pre-clinical and clinical approaches. Lastly, retrospective studies have indicated an anti-cancer/anti-metastatic benefit for drugs targeting the renin-angiotensin-system (RAS), which are commonly used to reduce hypertension and arterial blood pressure[191,192] (recently reviewed here[193]). Interestingly, the RAS inhibitor losartan was shown to not only provide anti-hypertensive but also anti-fibrotic benefits by decreasing ECM levels and thereby reducing physical stress, decompressing blood vessels and improving interstitial flow and the delivery and performance of therapeutics into the tumour tissue[194,195] (see also Box 1). Similarly, we have previously demonstrated that transient intervention with the vasodilator Fasudil in a preclinical model of pancreatic cancer decreased both fibrosis and metastasis while increasing vascularisation and improving chemotherapy performance[196]. Furthermore, targeting the ECM component hyaluronic acid using PEGPH20 was shown to normalise interstitial fluid pressure and to improve vascularisation and chemotherapy performance[197].

Extensive clinical data on large prospective patient cohorts using fluid-targeting agents are still sparse and therefore, further research will be required to determine



*[G] : see Glossary, page 26

the actual effect, mechanism of action and optimum timing for intervention as well as those cancer patient groups that will predominantly benefit from fluid-targeting agents.

**Intravascular arrest and extravasation**

Successful intravascular arrest and extravasation are also subjected to flow-mediated forces before metastatic outgrowth and use similar ancillary mechanisms to co-opt native cell types or fluid flow to facilitate colonization of secondary sites. Metastatic onset depends on a plethora of molecular programs[198] but also relies on successful intravascular arrest (or intravascular growth[199]) of CTCs before metastatic extravasation. Two main behaviours, that are not mutually exclusive, are responsible for the intravascular arrest of single or clustered CTCs that precedes metastatic outgrowth: (i) physical occlusion in microvessels or capillaries, as observed for instance by intravital imaging of human lung adenocarcinoma or melanoma CTCs in mice brains [102] or (ii) active adhesion of CTCs to vessel walls described in various *in vitro* and *in vivo* models [14,200–202]. In some cases, as for instance in the rat liver, physical occlusion cannot fully account for the efficient extravasation suggesting that active adhesion between CTCs and the vascular wall, upon CTCs physical trapping, is still required for extravasation and successful metastasis[101] (Fig 3).

From a biophysical point of view, Bell's model **[G]** provides an optimal model for describing the interaction and adhesion between CTCs and the endothelium under flow[203,204]. Such model takes into account the external fluid force as well as homogeneously distributed adhesion receptors binding the vessel wall (i.e. the number of physical contacts and the adhesion strength of contact between CTC and endothelial cell). When chemical bonds such as ligand/receptor interactions are subjected to external forces (including fluid flow), their adhesion energy has to overcome the energy of the fluid that is displaced. This model has widely been used to dissect the molecular machineries at play during leukocytes attachment to vessel walls[205] but the physico-chemical requirements for CTC attachment allowing adhesion to overcome shear remain largely unclear.

Interestingly, *in vitro* studies have suggested that CTCs hijack the arresting and transmigration strategies used by leukocytes during rolling[206] and diapedesis **[G]**. Rolling allows leukocytes to engage adhesion and thus to reduce their velocity in the flow. Studies using microfluidics showed that rolling of CTCs is mediated by the



*[G] : see Glossary, page 26

interaction between selectins at the surface of the endothelium and various glycoproteins at the surface of CTCs of different origins[207–210]. Receptor activation and flow-mediated endothelial stiffening[211] in the case of leukocyte rolling (due to flow pushing on the arrested cells – pulling on adhesions) will then trigger endothelial cell-cell junction opening and favour diapedesis[212]. CTCs might exploit similar flow-dependent mechanisms, in which they would adhere to the vessel walls by first being slowed down by weak adhesion force with fast activation rate. This initial transient interaction can provide time to activate and engage receptors with slower activation rate but higher adhesion force[5,205,213]. We recently dissected the molecular machineries at play during the arrest of murine mammary CTCs *in vitro* and *in vivo* and identified CD44 and ITGB1 as key mediators of CTCs arrest, by counteracting shear detaching the cells from endothelium as described by Bell's model[14,201]. Such stable intravascular arrest significantly underpins successful metastatic extravasation[201]. Whether the forces in the blood favour CTC rolling or catch-bond **[G]**-like adhesion remains to be studied[214]. The emergence of new quantitative tools to probe forces during CTC extravasation (Table 2, Supplementary Information S1 and Table 3) allows us now to document the flow-dependent intravascular arrest of CTCs at high-spatiotemporal resolution[215,14]. In addition, intravascular arrest, whether it is through physical trapping or active adhesion, is likely to be one of the most deleterious steps for CTCs: Cells must resist shear stress while simultaneously establishing stable engagement with the endothelium[178,216]. Upon adhesion, CTCs have a finite adhesion force (i.e. the force strength of the engaged adhesion receptor) to the vessel wall. Thus, CTCs are engaged into a tug of war between their limited adhesion strength and the force that the flow exerts on them with two possible outcomes: (i) if the CTC adhesion force is weaker than the shear stress: the CTC cannot adhere or is washed away; (ii) if the CTC adhesion force is equal or stronger than the shear stress: cells are able to arrest. In the context of intravascular injection of mammary carcinoma cells in zebrafish and mouse brain metastasis models, shear stress of 5 to 7 dyn/cm$^2$ favour CTC arrest and thus colonization[14]. Further work will be required to demonstrate that such values are valid in the context of other organs or cell types".

In addition to impacting the ability of CTCs to arrest in certain vascular regions, fluid forces influence the fate of arrested CTCs. Indeed, shear forces challenge their



*[G] : see Glossary, page 26

survival at potential colonization sites and are likely to detach already-adhered CTCs from the endothelium[14]. Arrested CTCs were found to experience higher shear forces once arrested[217], and therefore must develop adhesive molecular machineries to efficiently resist such forces for successful extravasation[14,201,215]. Intravital imaging of arrested tumor cells in mouse models of brain metastasis revealed that high shear forces can be deleterious to arrested CTCs[102]. In addition, intravital imaging of melanoma cells in mice lungs revealed that high shear forces can fragment arrested tumor cells[13], which will eventually recruit phagocytic cells that can influence metastatic onset[158]. Interestingly, arrested CTCs face mechanical constraints that are comparable to the ones impacting tumor cell invasion at primary tumours, and could thus potentially suffer from similar cell deformation-related events that have been extensively studied recently in confined spaces[218]. Such stresses, that alters organelle morphology[219–221] and impacts gene expression and cell survival *in vitro* (Table 1), have recently being modeled using microfluidic approaches and could be conveyed by shear forces at sites of intravascular arrest and whether they are at play during the intravascular arrest of CTCs, especially *in vivo* (Table 2 and Supplementary Information S1). As intravascular arrest, which is dependent on precisely balanced force interactions between CTCs and the endothelium, is a critical step in cancer metastasis, it may be particularly suited for therapeutic targeting. For example, fluid-targeting agents, such as beta blockers or RAS inhibitors as described before could be used to manipulate vascular profiles to detach already adhered CTCs from the endothelium. In addition, anticoagulants may serve to eradicate local pro-metastatic environments within the vessel lumen to impede initial CTC arrest, while also interfering with the interaction of CTCs and platelets in transit thereby (re-)exposing CTCs to shear stress and collision".

Mechanistically, extravasation of CTCs is thought to mirror immune cell extravasation[211], but the contribution of shear forces to this step remains elusive. Whether blood flow forces modulate such step *in vivo* remains unknown. The development and validation of new animal models and imaging techniques will allow us to document the contribution of flow forces to metastatic extravasation in more detail (Table 3). For example, *in vivo* CAM assays (Table 2 and Supplementary Information S1) show that cancer cell extravasation can involve cell protrusion (invadopodia), acto-myosin contractility and secretion of matrix metallo-



*[G] : see Glossary, page 26

proteinases[222,223]. Recent progress in intravital imaging models and technologies (Table 2, and Supplementary Information S1 and Table 3) now also allows us to study precise signaling dynamics that happen during cancer cell extravasation as has recently been shown for the activation of Src kinase upon the arrival of metastatic pioneer cells in the liver in a murine model of pancreatic cancer[224] (Fig. 3).

Recent data suggest that shear forces can stimulate non-tumor components such as platelets (observed with shear values of 7 to 8 Dyn/cm$^2$)[173] and endothelial cells (observed with shear values of 5 to 7 Dyn/cm$^2$)[14].Indeed, permissive flow velocities ( corresponding to ~500 µm/sec) allow endothelial cells to migrate intra-luminally in the presence of arrested CTCs in zebrafish or in mice [225,202,14] (Fig.3). They progressively enwrap arrested CTCs and preserve or re-establish normal perfusion while expelling metastatic CTCs that survived detrimental shear forces[14]. Interestingly, similar flow-dependent mechanisms had been described in mice for removing blood clots from blood vessels . Although the impact of shear forces on tumor cell dormancy has never been studied, it would be interesting in the future to determine whether perivascular niches[228] that provide a chemo-protective environment for dormant tumor cells[229] originate from cells that had extravasated through flow-dependent endothelial remodeling[14] and could thus be localized based on flow profiles. In the context of lung metastasis (Fig.3), shear forces rip fragments off arrested CTCs and promote competition between different immune cell populations that ingest the resulting cytoplasts **[G]**, thereby creating a pre-metastatic niche by favouring the recruitment of neutrophils, monocytes, macrophages and of rare lung-resident dendritic cells[13]. Resulting cytoplasts are ingested by several immune components that are recruited to that site and modulate extravasation of other CTCs. Alternative scenarios that require coordination between endothelial cells and CTCs favor metastatic extravasation. For example, extravasation of tumour cells through endothelial cell apoptosis was described *in vitro*[230] or through endothelial necroptosis **[G]** *in vivo* in a murine model of lung metastasis[231]. Also, tumour cells can proliferate intra-luminally to create massive emboli that are surrounded by endothelial cells but that stay bound to the normal vessels[199,225] (Fig.3). In conclusion, in addition to impacting the fate of CTCs, fluid forces are thus capable of indoctrinating stromal components that are likely to shape the metastatic success.

## Conclusions and perspectives



*[G] : see Glossary, page 26

The fate of tumour cells that are *en route* during metastasis and of tumour-associated factors is thus tightly controlled by bodily fluids and associated mechanics. Multiple challenges remain. Fluid displacement within tumours, and their underlying forces, is very likely to increase the dissemination of detectable tumour-associated factors that might hold a strong prognostic potential. While the impact of these forces on drug delivery has been studied extensively[23] (Box 1), we believe that additional studies are required to further understand how they promote tumour dissemination potential. Further work is also needed to address whether shear forces and fluid mechanics modulate the secretion and dissemination of tumour-secreted factors, and thereby the formation and location of PMNs, and of metastatic foci from distinct tumours. It is likely that flow routes and shear forces influence the arrest sites of soluble molecules and EVs alike, since they have also been shown to affect the internalization of small-scale synthetic nanoparticles by endothelial cells *in vitro*[232,233]. In addition, fluid shear stress promotes the efficacy of ROS-generating anti-cancer compounds, such as doxorubicin or cisplatin, which may provide an opportunity to exploit fluid mechanics towards targeted CTC apoptosis[119]. Organotropism and tumor-type distribution of secondary tumours could result, for example and in addition to other molecular factors, from distinct flow profiles and vessel architecture within and between organs. The combination of animal models with intravital imaging technologies at unprecedented spatio-temporal resolution[214] is likely to facilitate a significant leap towards the understanding of PMNs priming. While the clinical relevance of the metastatic *detour* from lymph nodes to distant organs remains to be determined, it will certainly impact therapeutic decisions in patients. Myeloid populations can sense tumour-derived material that is either transported or shed by fluid flows[73]. Besides, shear forces might tune such immune recognition of flowing tumour cells. A better understanding of how immune cell populations compete and are recruited at metastatic sites by fluid-borne material could lead to the design of refined anti-metastatic immunotherapeutics. Similarly, strategies aiming to reduce flow-mediated pro-metastatic endothelial remodelling **[G]** should be explored in the future. Anti-metastatic preventive interventions could be explored based on metastasis predilection sites, which would target regions with permissive flow profiles. Finally, whether clusters of CTCs are better equipped to co-opt other blood components or to adapt to shear forces during transit and to vascular constraints at metastatic sites remains to be demonstrated. Recent improvements in sensitivity of liquid biopsy



*[G] : see Glossary, page 26

analysis now allow to probe for minimal residual disease where metastatic foci or residual tumor cells (post-therapy) cannot be detected using conventional imaging techniques[234]. If combined with specific detection, within CTCs, of fluid-dependent biomarkers, clusters or interaction with relevant blood components (platelets or neutrophils), these methods are likely to boost the predictive and monitoring power of liquid biopsies. It is thus increasingly obvious that metastasis should be studied not only from a biochemical and genetic perspective, but also from a biomechanical angle that reveals how flow-associated forces influence metastasis. Such efforts could improve our understanding while providing new means to improve its detection and eradication. Further work is thus needed to determine whether fluid mechanics can potentially be used for a better targeting of anti-metastatic strategies in future perspectives.



*[G] : see Glossary, page 26

## Figures

**Figure 1: Fluid routes orchestrate the metastatic cascade.**

**A**. Metastatic dissemination is a non-random process based on biological and mechanical cues. Circulating tumor cells use blood and lymphatic circulation (yellow arrows) to reach distant organs, according to the anatomic plan and corresponding vascular pathways. Blue, Red and Purple arrows indicate common metastatic patterns respectively for colon, breast and pancreatic cancer. **B**. Interstitial flows allow tumor cells and tumor-shed factors to reach these circulatory systems in the primary tumor microenvironment (***1***). They will disseminate in the lymphatic circulation; seed draining lymph nodes and colonize local blood vessels (***2***). Ultimately, tumor-shed factors will prime pre-metastatic niches in target organs where disseminating tumor cells will grow as metastatic colonies upon hematogenous dissemination. **C.** Table describing flow parameters in human organs and various animal models.

**Figure 2: Intravascular environment of circulating tumor cells**

Circulating tumor cells experience several types of flow (see also Table 1) and interact with themselves or with blood components that would impact their metastatic potential. As single cells, they are more vulnerable to shear stress (***1***). They develop strategies to cluster or interact with other blood cells that are likely to protect or eliminate them, thereby shaping metastatic fitness (***2***).

**Figure 3: Tumor cells extravasation is a flow-dependent process.**

Extravasation of CTCs takes place mostly in capillary beds, where cells are able to arrest, either by occlusion (***1),*** despite obvious deformability potential ***(1'***), or active adhesion with the endothelial cells (***2***). CTCs that are stopped in the circulation will suffer from higher shear stress that can lead to cell fragmentation (***3***) and also subsequent modification of vessel permeability. Then, several extravasation scenarios are plausible. Cancer cells could undergo diapedesis (***4***), be extruded through flow-dependent endothelial remodeling (***5***), or grow intravascularly to form intraluminal metastasis (***6***). Some cancer cells will remain dormant for years to decades. Inbox : representative values of duration of the mentioned steps (Copyright and Permissions to be added) Images in 1 are reproduced with permission from Warren et al. (eLife 2018) and are the authors' own images. Images in 1' are



*[G] : see Glossary, page 26

reproduced with permission from Au et al. (PNAS 2016). Images in 2 are reproduced with permission from Entenberg et al. (Nature Methods, 2018). Images in 3 are reproduced with permission from Headley et al. (Nature 2016). Images in 4 are reproduced with permission from Leong et al. (Cell Reports 2014). Images in 5 are reproduced with permission from Follain et al. (Dev Cell 2018). Images in 7 are reproduced with permission from Ghajar et al. (2013).



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## Glossary

**Solid stress** is exerted by the solid constituents of a tissue and accumulates within solid structural components (i.e., extracellular matrix, tumor and stromal cells) during tumor growth.

**Margination** is a physical process that brings the cells or vesicles close to the endothelial wall.

**Exomeres** are a recently-described type of extracellular vesicles that are characterized by a low size (~35 nm) and an absence of membrane. They are enriched in metabolic enzymes and hypoxia, microtubule and coagulation proteins.

**Oncosomes** are a sub-type of large membrane-derived extracellular vesicles (microvesicles) secreted by cancer cells and which transfers oncogenic molecules to other cells.

**Viscoelasticity** defines a material that exhibits elastic and viscous behavior when placed under stress.

**Inertia** indicates to which extent cells and other material can maintain their motion against the flow.

**Fluid-structure interaction** occurs when the fluid deforms a physical structure, which will modify the fluid flow in turn.

**Metastable** refers to a state of least energy until more external energy is added to the system. This state appears stable although it is in theory unstable and capable of changing to a more stable state.

**Ramification** occurs in the vasculature when a stem vessel is branching out into two smaller-sized ones.



*[G] : see Glossary, page 26

**Fluid** is a substance devoid of rigidity. It continuously deforms and do not resist to shear stress applied to them.

**Viscosity** measures the resistance of a fluid to deformation at a given rate and quantifies the frictional force generated by layers of fluids that are in motion. Gases, water and other liquids are considered as Newtonian fluids because they display a linear correlation between shear stress and the rate of deformation. When fluids display a nonlinear relation between stress and deformation rate, they are considered as non-Newtonian: this is the case for blood.

**Shear stress** is a pressure that creates deformation when moving tangentially to a surface. For example, shear stress is generated when fluids flow over an endothelial surface.

**Interstitial fluids** are present around cells originating from blood capillaries leakiness. They bring nutrients to the cells and flow towards lymphatic draining giving raise to interstitial fluid flow.

**Tumor interstitial fluid pressure** is the hydrostatic pressure in the cellular interstice. In solid tumors this pressure is often due to vessel leakage and lymphatic drainage.

**Poiseuille flow** describes the velocity in a laminar regime for a viscous fluid moving in a cylindrical tube has a quadratic distribution.

**Flow types** are of two types of flow, laminar or turbulent. These two flows are dependent on the value of the dimensionless Reynolds number Re (see Table 1).

**Laminar flow:** In fluid dynamics, fluid particles transported in a laminar flow follow smooth directions in layers, with little or no mixing. Laminar flow is opposed to **turbulent flow** that is characterized by chaotic changes in pressure and flow velocity that create mixing of the flow paths of displaced particles.

**Hydraulic conductance** expresses the capacity of a porous material to allow fluid to cross under the effect of pressure differences.



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**Reynolds Number** is a dimensionless number proportional to fluid velocity V, density $\rho$, the displacement length L and the viscosity $\eta$. The value found for the Reynolds number traduces the transition between laminar and turbulent happens when the Reynolds number reaches values over 1000.

**Darcy's law** is a physical law, which in the context of cancer progression is linked to interstitial fluid and to hydraulic conductivity of the environment arising to a convective fluid displacement.

**Navier-stokes equations** are partial derivative equations describing in time and space the velocity field and the pressure of a fluid

**Bell's model** is a statistical model taking into account the chemical rates of formation and dissociation between ligand and receptors as well as the statistical distribution of these ligand-receptor pairs on two cell surfaces when external forces are applied.

**Catch bonds** are receptors-ligand interactions whose strength and lifetime increase under applied force.

**Circulating tumour cells** (CTCs) have left their primary tumour and entered the blood or lymphatic circulation (intravasation). They are exposed to a new fluid environment, which they co-opt to survive and to travel throughout the body.

**Convective flow** is a flow directed towards a gradient. In the case of solid tumors this gradient is a pressure gradient.

**Extracellular vesicles** (EVs) serve as cargo for the transfer of nucleic acids, proteins or lipids to distant sites. Their membrane composition provides a fingerprint for the targeted priming of specific organs.

**Liquid biopsy** involves the collection of non-solid tissues, such as blood, and screening for disease-related parameters, such as circulating tumour cells. In



*[G] : see Glossary, page 26

contrast to biopsy of solid internal tissues, liquid biopsy is minimally invasive and suited for repeated assessment in the same patient.

**Lymphangiogenesis** is the remodelling of existing lymphatic networks to give rise to new lymphatic vessels. VEGFC/D has been shown to play a prominent role in lymphangiogenesis induction and is secreted predominantly in the tumour periphery.

**Pre-metastatic niche (PMN)** is a new paradigm for the initiation of metastasis. Here, the pre-metastatic microenvironment is seeded/educated by tumor-secreted factors, which induce phenotypes that can help secondary tumor development.

**Soluble factors** can include protein ligands or extracellular matrix-modifying proteins that are secreted by tumour cells to induce the remodeling of distant sites for facilitated metastatic seeding

**Diapedesis** is the transmigration process by which circulating cells exit the blood stream either using the transcellular (through endothelial cells) or the paracellular (between endothelial cell through cell-cell junctions) routes.

**Endothelial remodeling** represents the ability of these cells to sense and respond to stimuli such as fluid flow, shear stress, and trafficking of immune cells. They remodel their contact adhesion to move towards specific reorganisation leading to flow homeostasis.

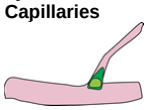
**Cytoplasts** are nucleus-free portions of cells that are released from arrested circulating tumor cells when facing high shear forces.

**Necroptosis** is a process which induces cell death through controlled necrosis.

**Thrombocytopenia** is a condition characterized by low platelets counts.



*[G] : see Glossary, page 26

| Fluid system | Properties | Shear Dyn/cm$^2$ | Velocities mm/s | Reynolds Number [G] | Contents | Biophysics and biomechanical forces | Cell Behavior / Phenotype |
|---|---|---|---|---|---|---|---|
| **Interstitial fluid**  | - Laminar flow<br>- Flow driven by gradient of interstitial fluid pressure (high in tumour core, low in periphery)<br>- High density of circulating objects | 0.1-1[5,235,236] | 0.001-0.004[38–40,237] | 0.00005-0.0002 | N/A | Darcy's model (convective and diffusive flow)<br>$Q = KA\frac{\Delta P}{\mu L}$<br>The flow Q of an incompressible fluid with viscosity η exerted by a loss in pressure ΔP through a porous material of area A possessing a hydraulic conductivity K. | - chemokine gradients enhance tumor cell migration[238]<br>- reduced tumor cell migration due to downregulation of MMPs[239]<br>- increase tumor cell invasion[42] and promote amoeboid phenotype motility of breast cancer cells[43]<br>- over expression of integrins, matrix metalloproteases (MMP), and ECM proteins [240,241]<br>- alteration of the expression of cytoskeletal proteins [242,243]<br>- epithelial-to-mesenchymal transformation (EMT), is characterized by a switch from keratin to vimentin intermediate filament expression. EMT results in cells with increased migration, invasion and dissemination potential [5,244] |
| **Lymphatic system**  | - Laminar flow<br>- Pulsatile with low amplitude at low frequency<br>- Flow driven by viscosity<br>- Low density of circulating objects | 0.64-12[19] | 0.02-1[245–247] | 0,002-3,3 *1E-5 | Cells (e.g. immune cells, tumour cells), tumour-derived acellular material | Navier-Stokes equation<br>$\frac{\partial u}{\partial t} + (u.\nabla)u = -\frac{1}{\rho}\nabla p + \nu\nabla^2 u$<br>$\nabla V = 0$<br>Where ρ is the density, ν is the viscosity, u the velocity vector and p the pressure. | - promote cell migration and motility in a YAP1-dependent manner[139] or to support cell division in a TAZ-dependent manner[103,140,248] |
| **Hematogenous sytem: Arteries**  | - Flow driven by cardiac pumping<br>- Pulsatile with high amplitude at high frequency<br>- Laminar to turbulent<br>- High density of circulating objects<br>- High velocity | 4-30[249–251] | 100-500[252–257] | 1-5000 | Platelets 150 to 350 billion cells/L<br>Red blood cells 4.7 to 6.1 million cells/μL<br>Neutrophils 5 × $10^9$ cells/L<br>Eosinophils 4 × $10^7$ cells/L<br>Basophils 4 × $10^7$ cells/L | Navier-Stokes equation (as above) with included fluid-structure interaction<br>Considering distensibility D and area A<br>$D \cong \frac{1}{A}\frac{dA}{dp}$<br>And the mass conservation law<br>$AD\frac{\delta p}{\delta t} = -\frac{\partial Q}{\partial x}$ | - even if shear stress resistance is a conserved property in tumor cells , it affects cell viability[258]<br>- High shear stress causes necrosis in over 90% of CTCs[80]<br>-Associates to Platelets reduce anoikis and promote metastasis by activating YAP1 signaling<br>- CTCs will increase the expression of adhesion protein (CD44, integrins)[240,260–262] allowing adhesion on endothelial walls when flow velocity is below 600μm/s<br>Correlation between cell death and increasing shear stress[122,263,121,119] |
| **Hematogenous system: Veins**  | - Flow driven by cardiac pumping<br>- Laminar flow<br>- Pulsatile with low to medium amplitude at medium frequency<br>- High density of circulating objects<br>- Slow velocity | 1-4[249,250] | 1-200[264,265] | 0.006-100 | | | |
| **Hematogenous system: Capillaries**  | - Flow driven by cardiac pumping<br>- Pulsatile with low to medium amplitude<br>- Laminar<br>- low density of circulating objects<br>- Slow velocity | 10-20[249,250] | 0.01-1.5[266–268] | 0.003-0.004 | | | |

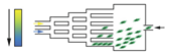
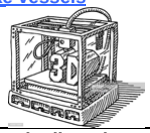
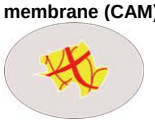
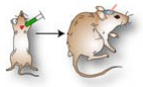


*[G] : see Glossary, page 26

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

## Table 1: Key features of body fluid systems involved in cancer cell transport

## Table 2 : Models to track intravascular dynamics of CTCs

| Model | Approach | Advantages | Disadvantages |
|---|---|---|---|
| **Microfluidics**  | Cancer cells can be exposed to tuneable shear stress. Channels can be coated with other cell types or substrates to study molecular and cellular interactions of CTCs in transit [55,80,139,140,200,201,208,269–272] | - fast assay<br>- medium to high throughput<br>- variable pricing depending on setup<br>- uncomplicated integration of live cell imaging | - low fidelity, ex vivo assay<br>- lack of microenvironment / artificial microenvironment |
| **3D printing of human-like vessels**  | Vascular casting of vessel-like networks that can be perfused and lined with endothelial cells, using for example carbohydrate glass[273] or biocompatible hydrogels[274] | - control of network geometry<br>- endothelialization<br>-cell-compatible materials | - technology that requires a specific expertise and material |
| **Chorioallantoic membrane (CAM)**  | Uses the blood vasculature on the yolk sac of the developing chick embryo. Transplantation of tissues on the CAM or i.v. injection of cancer cells around embryonic day 12 for studies of intravasation, CTCs and extravasation[222,223,275–277] | - fast assay<br>- medium throughput<br>- low cost<br>- amenable to live microscopy | - embryonic vasculature may fail to faithfully recapitulate metastatic events in adult organisms<br>- no long-term tracking due to foreign tissue rejection in late-stage embryos<br>-<br>- requires exogenous labelling of any microenvironmental compartments |
| **Zebrafish**  | Cancer cells are introduced via microinjection (e.g. intravenously, pericardium, perivitelline space) at embryonic stages from 36 hours post fertilisation onwards[14,73,91,162,215,278–287] | - fast assay<br>- mid/high-throughput assay with improved fidelity over *in vitro* systems<br>- low maintenance costs<br>- transparency at young stages facilitates imaging<br>- growing toolbox of transgenic of microenvironment<br>- amenable to pharmacological or transient genetic manipulation | - embryonic vasculature may fail to faithfully recapitulate metastatic events in adult organisms<br>- lack of transparency and potential foreign tissue rejection and in adult fish prevent long-term tracking<br>- different vascular structure, anatomy and organisation compared to mammals/humans<br>- zebrafish genome duplication can hamper comparisons in gene functions between zebrafish and higher organisms |
| **Mouse**  | Very versatile platform: cancer cells can be introduced via injection (e.g. intravenous, intrasplenic, intracardiac) for short-term metastasis assays or genetically engineered mouse models of sporadic cancer formation can be used to study autochthonous CTC biology [13,55,102,158,224,288–305] | - assessment of CTC behaviour in their native environment<br>- high fidelity insights into CTC biology using of genetically engineered mouse models of cancer<br>- large number of transgenic lines to label the microenvironment<br>- potential for surgical interventions that are also performed in human patients<br>- amenable to short- and long-term pharmacological studies | - high maintenance and experimental costs<br>- induced/xenograft models can be less relevant<br>- intravital imaging requires surgery or the insertion of windows to access secondary sites/organs<br>- anesthesia for imaging may affect flow dynamics and fluid mechanics |
| **Patient CTC-derived xenografts (CDXs)**  | CTC isolation from whole-blood samples of patients via enrichment of epithelial cells and subsequent transplantation into mice[170,306–317] It is becoming evident that patient CTCs can also exhibit mesenchymal features, which may require an optimisation of the isolation protocol. Largely unexplored potential of applications in studying patient-specific response to biomechanical stress | - easily accessible via liquid biopsy **[G]** from all disease stages<br>- maintain similarity to original patient's disease and treatment sensitivity/resistance<br>- longitudinal sampling to assess pre- *vs* post-treatment/-surgery stages<br>- can be used in other models discussed above with genetic or pharmacological manipulation<br>- allows for personalised treatment approaches and can be linked to clinical outcomes | - high maintenance and experimental costs<br>- high involvement of ethical regulations<br>- rather low success rate in CDX generation<br>- clonality/heterogeneity of samples<br>- longitudinal sampling may be limited to patient tolerance and health status/disease stage<br>- currently mostly limited to introduction into immunodeficient models |



*[G] : see Glossary, page 26

| | | | |
|---|---|---|---|
| **Mathematical Models**<br>  | Numerical modelling focuses on the propagation, arrest and formation of thrombus by CTCs.<br>Modelling real human vascularisation and flow propagation in 3D[105].<br>Stiffness, Flowing properties and adhesion to vessel wall of CTCs[107].<br>Margination properties of CTCs with respect to erythrocytes[108,109].<br>Hemodynamic behavior of cluster of CTCs : rotation and tumbling during traveling[108]<br>Thrombotic events linked to CTCs crowding[110,111] | - Versatility and tunable parameters: flow rate, cell number, adhesive and viscoelastic properties of CTCs, etc...<br><br>- Predictive power that is out of reach with experimental conditions. | - Dependent on computing power and time.<br><br>- 3D modelling remains complex, cumbersome, time-consuming and costly<br><br>- Difficult to integrate existing models<br><br>- Absence of a specific platform dedicated to the study of fluid mechanics in cancer |

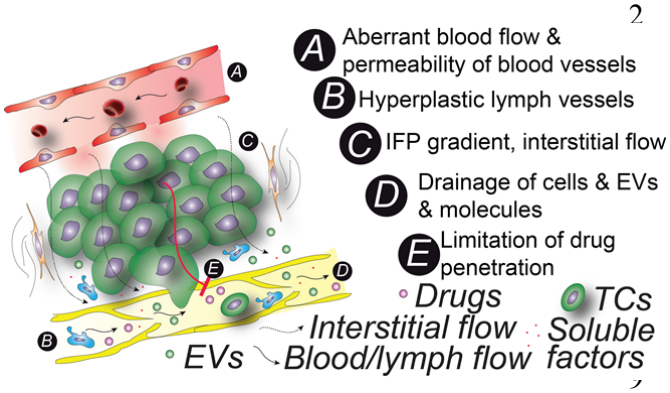
## Table 3 : Quantitative methods to probe forces associated with metastatic extravasation

| Method | Application | Advantages | Disadvantages |
|---|---|---|---|
| Optical tweezers for measuring hemodynamic forces *in vivo* | - *in vivo* measurement of forces on cells, such as red blood cells | - directly read out forces experienced by cells in the circulation<br>- high fidelity<br>- non-invasive | - low throughput, high cost<br>- specialised skill set and equipment required<br>- limited depth *in vivo*<br>- requires intravital imaging compatible animal models<br>- limited to forces ~100 pN |
| Förster Resonance Energy Transfer (FRET) biosensors | - spectrin repeat stress sensitive sensors[318,319] as well as actinin[319–321] and other molecular tension sensors[322–327] to directly measure mechanical forces or response adaptation thereto<br>- Biosensors of Src[328], FAK[329] or small GTPase signalling[330–333] to read out distinct intracellular signalling pathways upon biomechanical input | - single cell and subcellular readouts achievable<br>- reversibility of FRET biosensors allows detection of dynamic changes in biomechanical forces<br>- ongoing development to increase the range of signalling pathways to be interrogated<br>- compatible with *in vitro* and *in vivo* readouts<br>- longitudinal or continuous mapping of force changes and cellular responses | - can require advanced imaging and analysis setups<br>- gauging of fluorescence intensity/expression levels is important<br>- relatively low dynamic range requires careful calibration and experimental controls |
| Brillouin microscopy | - Measurement of intracellular[334] and extracellular[335] biomechanical properties<br>- combination with Raman imaging for mechanical mapping[336] | - label-free<br>- readout of forces at subcellular level<br>- can be used to assess hydration status and thereby infer interstitial fluid level/pressure | - Limited *in vivo* application<br>- very weak signal requires long acquisition times<br>- complex interpretation of Brillouin spectra for highly hydrated materials |
| Measurement of hemodynamic forces using intravascular beads | - measure flow rate and turbulence experienced by cells in vessels by tracking injected fluorescent beads[14] | - enables visualisation of flow rates and turbulence<br>- allows precise measure of flow velocities<br>- demonstrated in live animals | - limited to regions where beads are able to perfuse from site of injection<br>- invasive method<br>- requires automated image analysis |



*[G] : see Glossary, page 26

## Box 1: Interstitial fluid pressure (IFP) at primary tumor sites




Aberrant flow and permeability of intratumoural blood vessels often results in continuous plasma deposition into the extracellular space (**A**), which cannot be cleared or drained due to tumour-growth-induced compression of intratumoural lymphatic vessels. This leads to high interstitial fluid pressure within the tumour[35,337]. Increased lymphangiogenesis in the tumour periphery (**B**[338]) then creates a gradient of IFP (high intratumoural IFP and low IFP in the tumour periphery) with interstitial flow from the tumour centre to and beyond the tumour border (**C**).

Interstitial flow can promote the distribution of tumour-derived cells, EVs and molecules into the periphery (**D**), whereas high IFP in the tumour core has been shown to limit efficient drug delivery (**E**[23,34]). In vitro microfluidics models suggest that interstitial flow can also affect the mode of cell migration and the directionality of cancer cell migration[43] directly through inducing asymmetric focal adhesion distribution[339,340] as well as indirectly by guiding the differentiation of tumour-supporting stromal cell populations[44].

Recent approaches to mathematically model IFP for difficult-to-access sites, such as brain tumours[341], may provide further insight into the role of IFP in tumour progression, metastasis and response to treatment.



*[G] : see Glossary, page 26

**Table S1**: Applications of *in vivo* models to track intravascular dynamics of CTCs

| Model | Applications |
|---|---|
| **Microfluidics** | - detailed high-resolution monitoring of distinct steps of extravasation[200]<br>- isolation of live animal- and patient-derived circulating tumor cells (CTCs) from whole blood using microfluidics[55,269,270]<br>- mimic metastatic tropism via inclusion of secondary site - specific cells[271,272]<br>- simulate exercise-related shear stress on CTCs[80]<br>- dissection of signalling events that drive response to shear stress[139,140] or intravascular CTC arrest[201,208] |
| **Chorioallantoic membrane (CAM)** | - role of protrusion and invadopodia formation in extravasation and metastasis[222,223,275]<br>- assessment of extravasation efficiency[276,277] |
| **Zebrafish** | - several applications to identify the interactions between CTCs and endothelial cells that stimulate extravasation<br>- *in vivo* characterisation of biomechanical[14], cellular[162,215,278] and molecular mechanisms[279,280] that guide CTC arrest and subsequent extravasation in zebrafish embryos[281]<br>- live imaging of cancer cell survival under shear stress in the circulation[282]<br>- identification of pro-metastatic effects of shear stress via reactive oxygen species (ROS[283])<br>- testing of drugs that target CTCs[284]<br>- co-injection of cancer cells and immune cells to assess the interactions between CTCs in transit and the innate immune system[285]<br>- co-injection of cancer cells with patient-derived exosomes to demonstrate that exosomes can promote metastasis[286]<br>- intravital tracking of tumour-derived extracellular vesicles (EVs[73]) or zebrafish exosomes[91] to delineate targets that receive EV/exosome information<br>- introduction of *Casper* zebrafish model lacking melanophores and iridophores to study CTC behaviour in live adult zebrafish[287] |
| **Mouse** | - short-term and longitudinal *in vivo* CTC imaging in anesthetised animals through optical imaging windows in the lymph node[288], liver[224], lung[13,158], brain[102] or bone[289,290]<br>- miniaturisation of intravital microscopy setups[291] allows for mounting of imaging setup on the animals and intravital imaging of CTC behaviour in awake animals[342] thus avoiding flow artefacts caused by anaesthesia<br>- *in vivo* flow cytometry to quantify CTC abundance or clustering while in transit [55,292,293]<br>- allows visualisation of extravasation dynamics in target organs[224]<br>- introduction of FRET biosensors and biosensor mice (please see Box/Table 3 for more detail) to image molecular hallmarks of cancer that are known to drive cancer invasion and metastasis[294–296]<br>- intravital correlated light and electron microscopy (CLEM) for high-resolution assessment of cells and structures post-intravital imaging[297–299]<br>- optical clearing procedures further increase post-fixation imaging depth[300–304] |
| **Patient CTC-derived xenografts (CDXs)** | - transplantation of patient-derived CTCs into immunocompromised mice for small cell lung cancer[306], melanoma[307], non-small cell lung cancer[308], triple-negative breast cancer[309]<br>- *ex vivo* expansion of patient-derived CTCs prior to CDX generation allows for genetic manipulation, e.g. fluorescent labelling, CRISPR[310]<br>- applications to characterise patient tumour composition and progression[311–314] or to test and validate new treatment combinations for patients with chemoresistant disease[315,316] with recent emergence of longitudinal models[343]<br>- potential to isolate CTC clusters from patients to understand single-cell and population heterogeneity[170,317]<br>- largely unexplored potential of applications in studying patient-specific response to biomechanical stress |



*[G] : see Glossary, page 26

**Highlighted bibliography list**

**Wirtz et al. NRC 2011**

This review paper provides one of the first important discussions of the importance of how mechanical forces control interaction of cancer cells with their microenvironment, including blood flow, and how such forces are essential to the metastatic process.

**M. B. Headley *et al.*, *Nature*. 531, 513–517 (2016).**

This paper uses intravital imaging of metastatic dissemination in the lung and reveals that shear flow fragments CTC and therby generates immune-interacting intermediates that define efficiency and metastatic cell seeding.

**G. Follain *et al.*, *Dev. Cell*. 45, 33–52.e12 (2018).**

This paper provides the first demonstration that metastatic dissemination occurs in vascular regions with permissive flow profiles. It further shows metastatic extravasation is facilitated by endothelial remodeling, that is dependent on blood flow forces.

**M. Brown *et al.*, *Science*. 359, 1408–1411 (2018).**

This paper shows that tumor cells experimentally delivered to lymph nodes can efficiently disseminate by invading local blood vessels. Lymph node blood vessels are thus also used by cancer cells for efficient dissemination to distant organs.

**E. R. Pereira *et al.*, *Science*. 359, 1403–1407 (2018).**

This paper used photoconversion of tumor cells to track metastatic cells located in the lymp nodes and track their fate. It demonstrates that such cells efficiently leave lymph nodes via local blood vessels to seed distant organs.

**N. Aceto *et al.*, *Cell*. 158, 1110–1122 (2014).**

This paper shows that CTC can be found as clusters in the bloodstream that display increased metastatic potential. It further shows that plakoglobin mediates intravascular cluster formation through intercellular adhesion.

**Szczerba BM et al. Nature. 2019 Feb;566(7745):553-557**



*[G] : see Glossary, page 26

This paper shows that clusters of circulating tumour cells (CTCs), which are precursors of metastasis and are found within the bloodstream, are accompanied by neutrophils. Presence of neutrophils is associated with cell cycle progression of CTCs within the bloodstream, thereby promoting their metastatic potential.

**Y. Kienast et al., Nat. Med. 16, 116–122 (2010).**

This paper uses longitudinal real-time intravital imaging to provide a clear description of intravascular steps during brain metastasis formation. It shows that arrest of CTCs at vascular branch points of microvessels is a key step in metastatic progression and offers a very useful *in vivo* approach for tracking intravascular behavior of CTCs.

**Au *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* 113, 4947–4952 (2016).**

This paper shows that clusters of CTCs can successfully flow through vessel constrictions. CTCs reduce their hydrodynamic resistance by reorganizing into single-file chain-like clusters.

**Rizvi et al. Proc Natl Acad Sci U S A. 2013 May 28; 110(22): E1974–E1983.**

This study uses microfluidic approaches to shows that flow forces increase epithelial–mesenchymal transition of tumor cells and favor their motility and aggressiveness.

**Mitchell et al. Am J Physiol Cell Physiol. 2015 Dec 1; 309(11): C736–C746.**

This study shows that tumor cells are resistant to shear forces and display reduced apoptosis and necrosis when compared to normal cells. It further shows that such resistance is mediated by Lamin A/C that acts as structural component and facilitates survival.

**Warren et al. Elife. 2018 Jul 9;7. pii: e35800. doi: 10.7554/eLife.35800.**

This study highlights the application of intravital FRET (Förster Resonance Energy Transfer) imaging to characterise signalling dynamics that might be involved in cancer cell extravasation and arrival at secondary sites. The publication also characterises a novel software tool that can be used to correct for sample motion, a major challenge commonly encountered in intravital imaging due to animal respiration or heartbeat.



*[G] : see Glossary, page 26

**Weiss *et al*. *Invasion Metastasis*. 1981;1:126–135**

This study compared the metastatic rate of eight target organs with their arterial blood flow, in the context of colorectal cancer and esophageal squamous cell carcinoma. They observed that frequency of organ metastasis correlates with blood flow.

**Entenberg et al. *Nature Methods* 2018 ; 15 :1**

This study provides methodologies for stably tracking formation of lung metastases in a murine model. It allows to accurately track intravascular arrival of CTCs, extravasation, growth and progression to micrometastases.

**Zomer et al. *Cell 2015***

This study uses intravital imaging to track local and distant dissemination of EVs and demonstrates that EVs uptake shape metastatic fitness.



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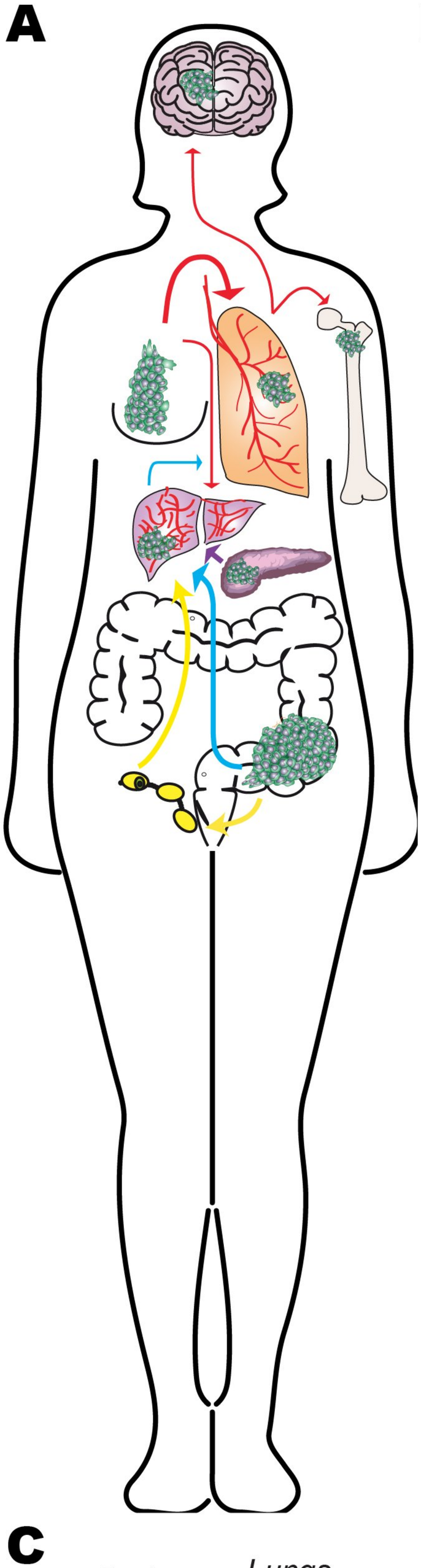
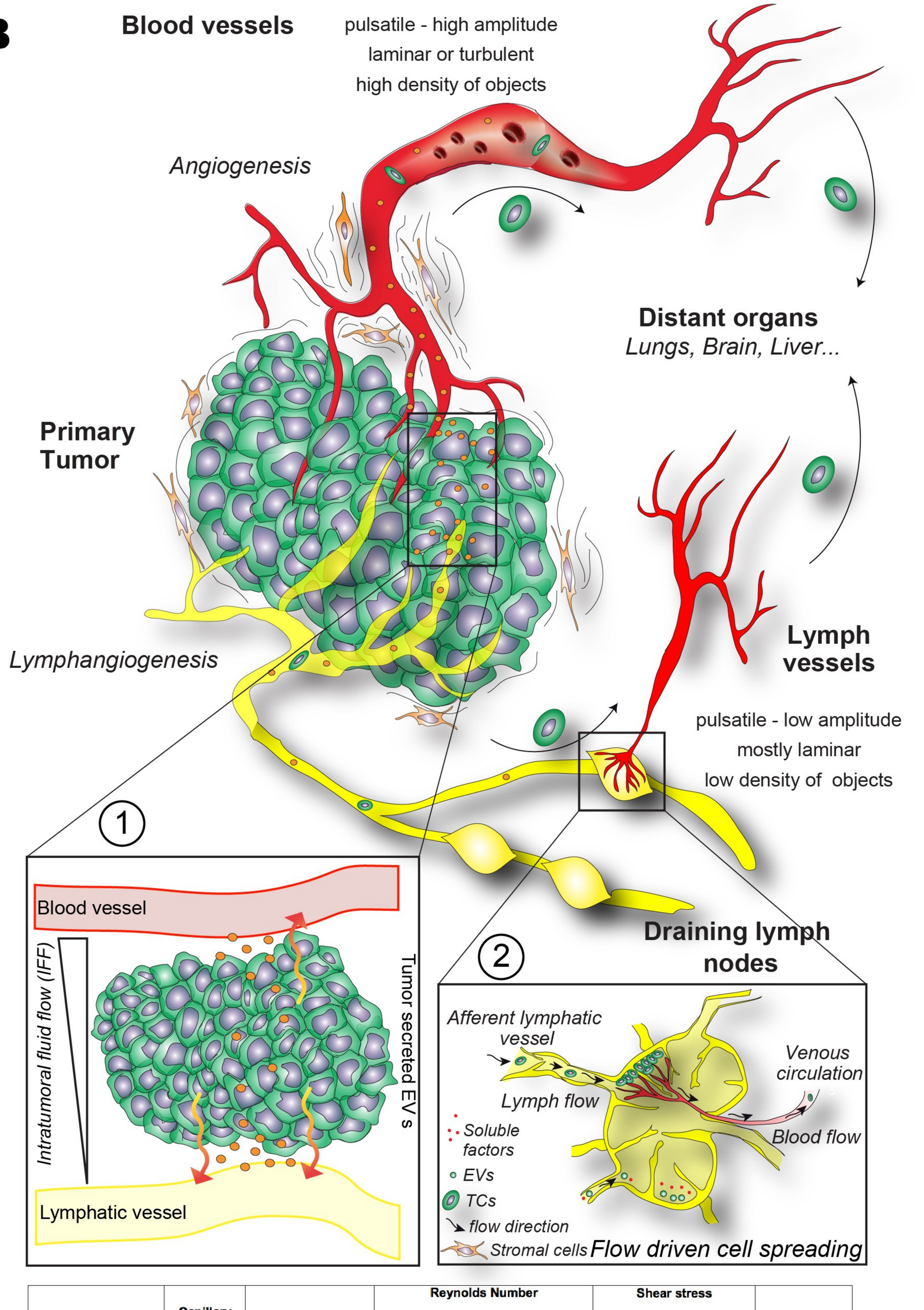
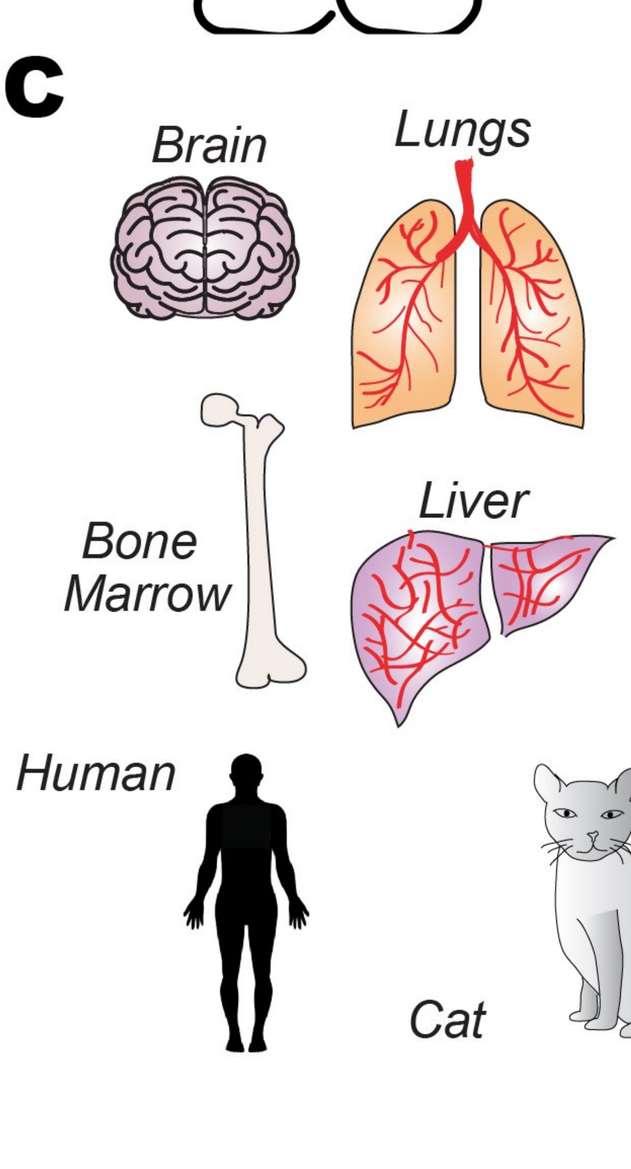
**A**



**B**




**C**




| Animal Model | Capillary diameter (μm) | Velocity range (mm/s) | Reynolds Number $Re = \frac{\rho V D}{\eta}$ (dimensionless) | Shear stress $\sigma = \eta \cdot \frac{\partial v}{\partial y}$ (Pa) | Refs |
|---|---|---|---|---|---|
| Human Brain | 6<ϕ<10 | 0.8<V<8 | 0.00135<Re<0.01688 | 0.4<σ<6.7 | 1–4 |
| Human lungs | 10<ϕ<90 | 1.8<V<2.3 | 0.00379<Re<0.0436 | 0.1<σ<1.15 | 5–7 |
| Human liver | 16<ϕ<10000 | 0.3<V<180 | 0.001<Re<379 | 0.09<σ<0.3 | 8–11 |
| Human BM | 8<ϕ<10000 | 0.23<V<75 | 0.00038<Re<158 | 0.037<σ<0.14 | 12–15 |
| Cat brain | 5 | 0.4<V<3.9 | 0.00042<Re<0.0041 | 0.4<σ<3.9 | 16 |
| Rat Brain | 2<ϕ<5 | 0.8 | 0.0003376< Re<0.000844 | 0.8<σ<2 | 17 |
| Mouse Brain | 4<ϕ<8 | <0.4 | 0.0003376<Re<0.000675 | 0.25<σ<0.5 | 4,18 |
| Zebrafish(2dpf) | 8<ϕ<28 | 0.3<V<2.5 | 0.00115091<Re<0.03356 | 0.024<σ<0.7 | 19–22 |

*N.B: Reynolds number and shear stress have been calculated using the following blood viscosity η density of blood ρ. Zebrafish: η=0.0022 Pa.s; Mammalian models: η=0.005 Pa.s; ρ=1055 kg.m⁻³.*

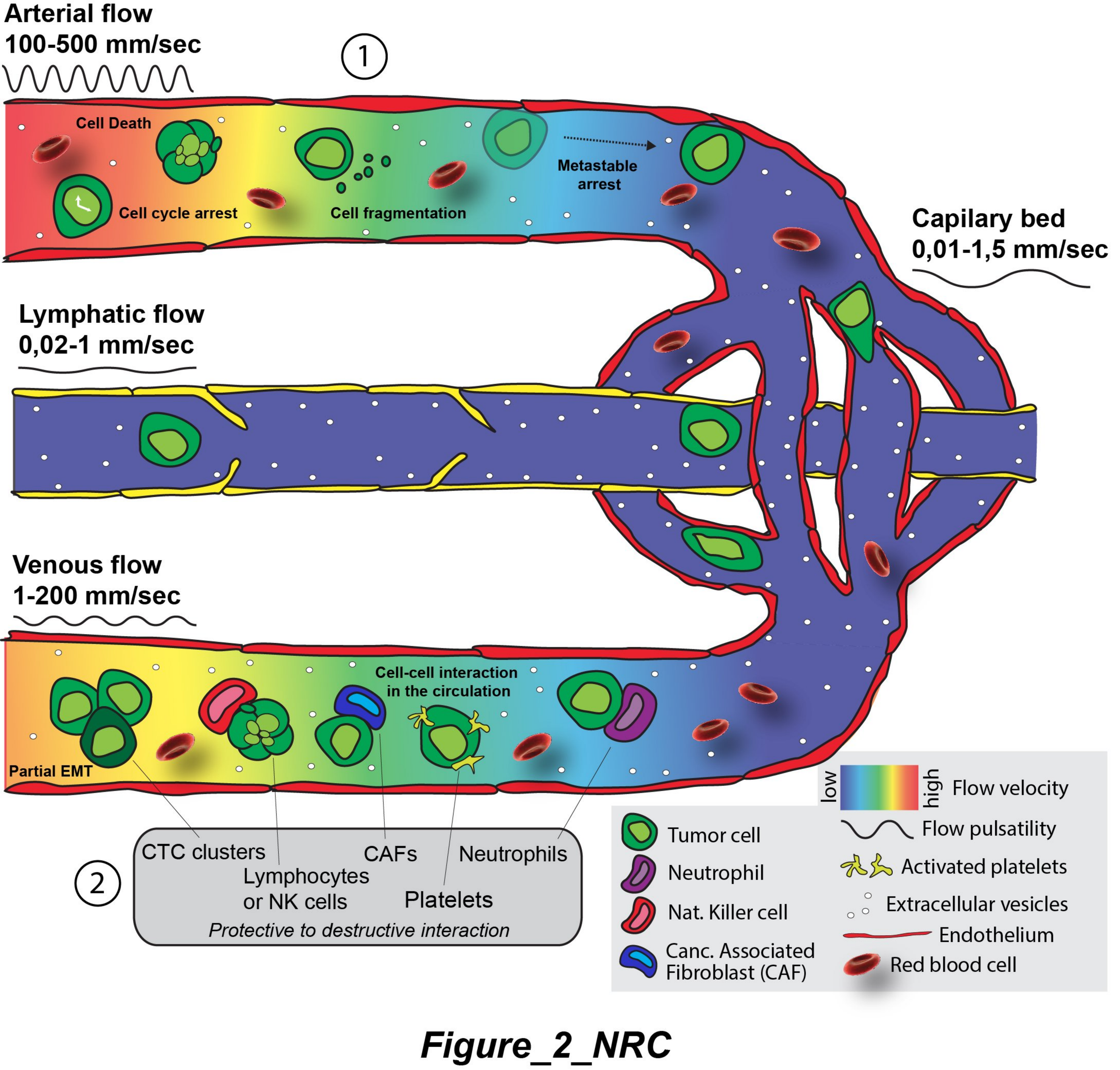
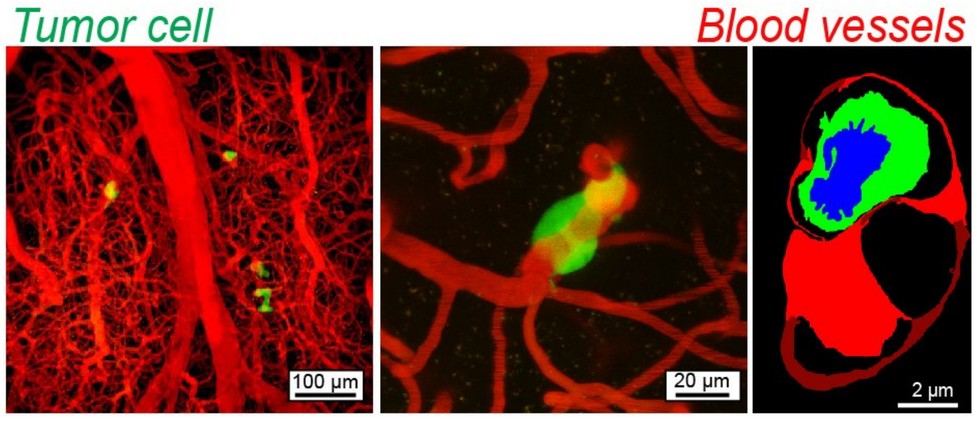

Arterial flow
100-500 mm/sec
1
Cell Death
Cell cycle arrest
Cell fragmentation
Metastable arrest
Capilary bed
0,01-1,5 mm/sec
Lymphatic flow
0,02-1 mm/sec
Venous flow
1-200 mm/sec
Cell-cell interaction in the circulation
Partial EMT
2
CTC clusters
Lymphocytes or NK cells
CAFs
Platelets
Neutrophils
Protective to destructive interaction
low
high
Flow velocity
Flow pulsatility
Tumor cell
Neutrophil
Nat. Killer cell
Canc. Associated Fibroblast (CAF)
Activated platelets
Extracellular vesicles
Endothelium
Red blood cell

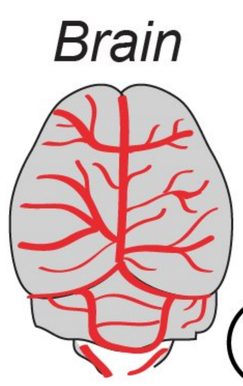
***Figure_2_NRC***

| | |
|---|---|
| Circulation | 6-10 min for CTC clusters<br>25-30 min for single CTCs |
| Extravasation | Starts few hours after arrest<br>2h<duration<24h |
| Outgrowth<br>Dormancy | 2h<duration>months,years |

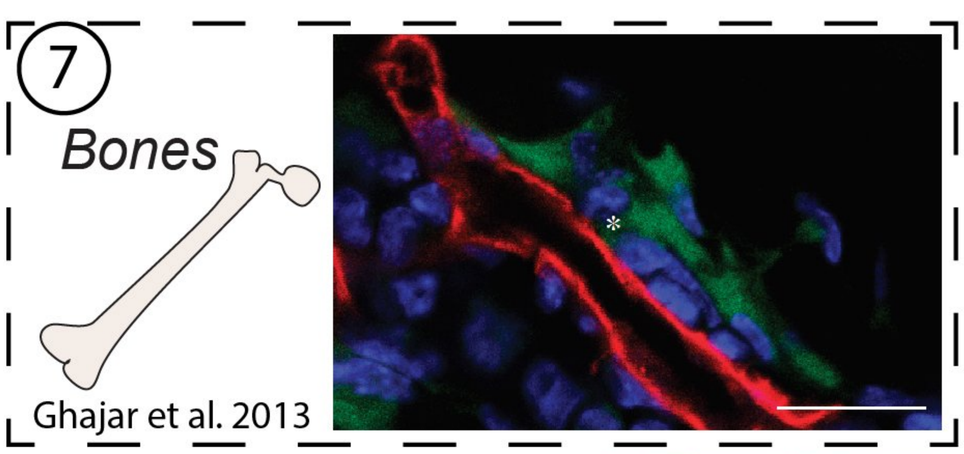
5



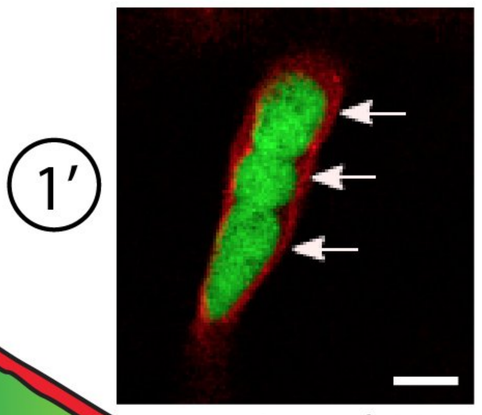

Follain et al. 2018




7



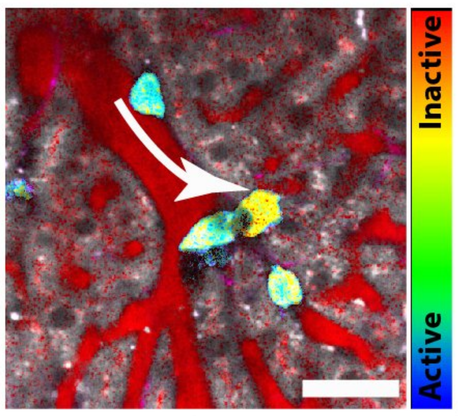

**Flow-dependent Endothelial remodeling**



1′

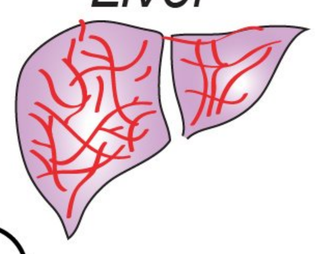
Au et al. 2016

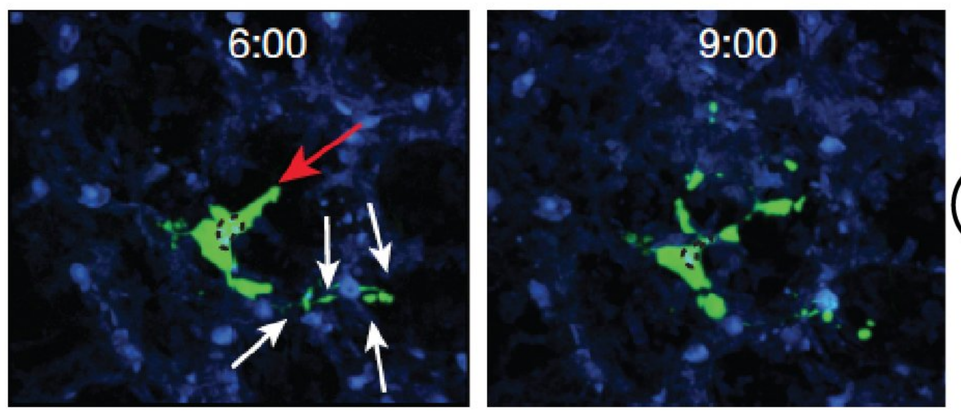



Warren et al. 2018

1

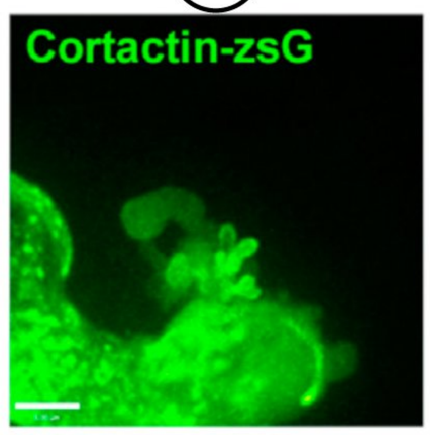


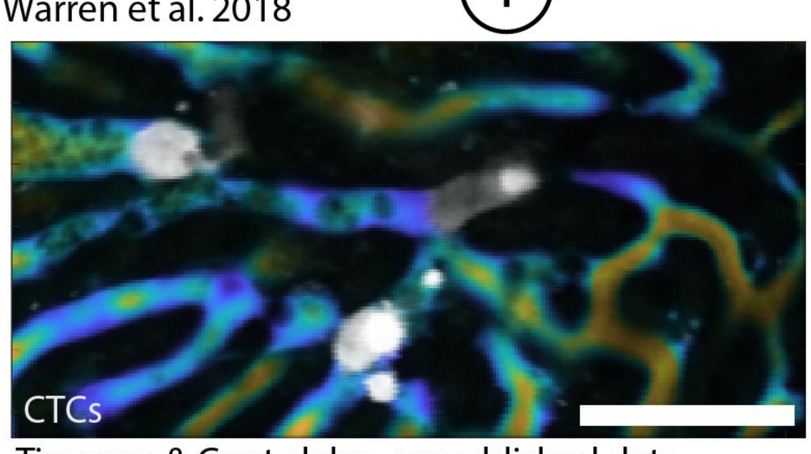




Headley et al. 2016

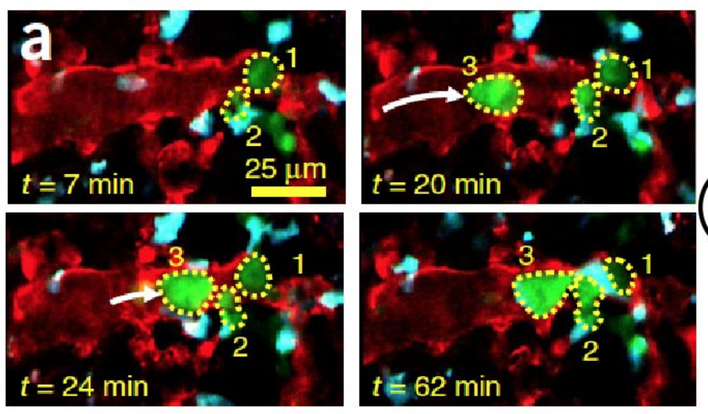
3

4

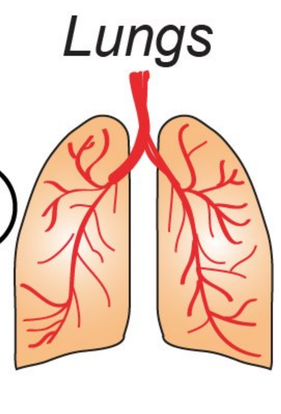


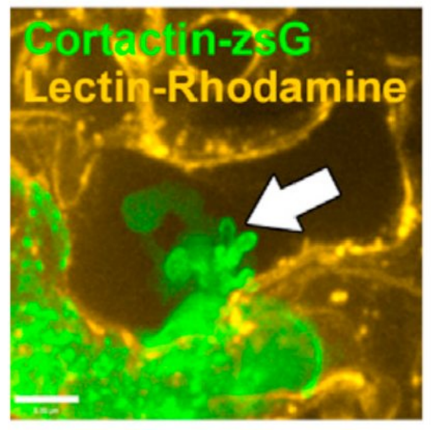




Timpson & Goetz labs - unpublished data




Entenberg et al. 2018




Leong et al. 2014

Tumor cell · Monocyte · Fibroblast · Activated platelets · Red blood cell · Endothelial cell · Extracellular vesicles

**Figure_3_NRC**