Platelet activity may be involved in tumor metastasis. The tumor cells, after detachment from the primary site, adhere to vascular endothelium at distant sites and proliferate. Platelets form aggregates with tumor cells in circulation, facilitating their adhesion to the vascular endothelium. Formation of platelet–tumor cell aggregates and their sequestration in various end-organs may result in thrombocytopenia. Certain tumor cell lines directly stimulate platelet activity, some by releasing platelet-aggregating material, a urea-extractable membrane component, others by release of cathepsin, and still others by undefined mechanisms. The direct effect of platelets on tumor cells may be of pathogenic significance. For example, platelet-derived factors stimulate growth of some tumors, whereas others increase vascular permeability and thus facilitate migration of tumor cells across the vessel wall. Lack of these platelet factors, as in thrombocytopenic animals, may indeed inhibit tumor metastasis. Arachidonic acid metabolism in platelets and the vessel walls may contribute to metastatic process. In particular, thromboxane A2 and prostacyclin generation capabilities appear to be important in modulating platelet–tumor cell deposition and growth. To alter the metastatic process, several preliminary trials of platelet-inhibitory agents have been performed. However, the results of these trials have been equivocal, perhaps related to nonspecific effects of these agents on arachidonic acid metabolism. Studies directed at specific pathways of platelet-vessel wall interaction on some tumors appear promising. These newer agents may be of therapeutic value in man.

The metastatic process is generally believed to involve several steps, including detachment of the cells from the primary tumor, spread of these cells into the bloodstream or lymphatic channels, systemic infiltration, and proliferation. Release of the tumor cells from the primary site into the circulation depends on several factors. Some of these factors were studied by Loetta and associates.2,3 The number and size of the cellular aggregates released into the circulation after injection of transplantable fibrosarcoma cells under various conditions were evaluated in mice. The tumor cell aggregates released in the circulation increased with time after injection of the tumor cells in the first 15 days. Trauma to the primary tumor was another factor predisposing to release of tumor cells. Both mechanical and chemical trauma resulted in an increased number of tumor cells singly and in large aggregates in the circulation. Following trauma, pulmonary metastases were also more frequent.

Necrosis of the primary tumor may also relate to the release of tumor cells in the circulation. When tumor undergoes necrosis, tumor cells detach from the primary site and are released. It has been shown in experimental animals that addition of necrotizing factor to the tumor cells results in separation of individual cells, which are likely to be released in the circulation.4 Furthermore, cells from the center of tumor adjacent to necrosis are much more likely to separate than those in the periphery.4 The role of blood vessels, both in and away from the tumor, may also influence the development of metastasis. The release of tumor cells is directly proportional to the density of large vessels at the primary site.2,3 The integrity of blood vessels distal to the primary tumor site also affects development of metastasis. Intact endothelium represents the first important barrier to the spread of tumor cells. Salsbury and associates5

From the Department of Pediatrics, Division of Hematology-Oncology, University of Florida College of Medicine, Gainesville, FL. Supported in part by grants from the Florida Affiliate of the American Heart Association and the American Cancer Society. Submitted April 27, 1983; accepted July 16, 1983. Address reprint requests to Dr. Paulette Mehta, Department of Pediatrics, Division of Hematology-Oncology, Box J-296, J. Hillis Miller Health Center, University of Florida College of Medicine, Gainesville, FL 32610. © 1984 by Grune & Stratton, Inc. 0006-4971/84/6301-0007$01.00/0.
were able to directly relate the antimetastatic potential of ICRF-159 to the strengthening of the blood vessels. ICRF-159, at concentrations much lower than those required to influence primary growth, prevented metastatic deposits. In animals pretreated with ICRF-159 and then injected with Lewis lung carcinoma tumor cells, no cells could be detected in the peripheral blood. In contrast, in control animals injected with the same tumor cells, but without ICRF-159 pretreatment, tumor cells were abundant in the circulation within 10 days after injection of the cells. Histologic analysis showed that the vascularity of the primary tumor site was greatly reduced and that the vascular endothelium was reinforced structurally. This suggests that the intact vessels form an important barrier to escape of tumor cells into the bloodstream.

The presence of tumor cells in the circulation does not necessarily imply eventual metastatic deposition or spread of disease. Once the tumor cells are released, subsequent distal deposition will depend on several factors. One of these factors is the intrinsic adhesiveness of the tumor cells to the endothelium. Adhesiveness may vary in different cell lines of the same tumor. For example, two variant cell lines from melanoma tumor were shown to have distinct patterns of adhesiveness. The adhesiveness of the cells to lung parenchyma in vitro correlates directly to the ability of tumor cells to be retained within the lung. Adhesiveness may also be affected by the relative electrostatic charges between the tumor cells and endothelium. The observation that neuraminidase decreases negative charge and increases adhesiveness of tumor cells to the endothelial lining suggests an important role of electrostatic charges in eventual development of tumor metastasis.8

ROLE OF PLATELETS IN TUMOR METASTASIS

Platelet-Tumor Cell Aggregate Formation

Platelet activity can promote deposition of tumor cells to the vascular surface. Wood and associates first suggested the association of platelet thrombus formation and tumor cell deposition.9,10 In their classic studies, the fate of V2 carcinoma cells injected into rat earlobe veins was examined. These investigators demonstrated that, shortly after injection, the cells adhered to the local vascular endothelium, and within a few minutes, were enmeshed within a thrombus. The critical role of cell attachment to the endothelial lining as an initial event in tumor cell metastasis was subsequently confirmed and defined by others.11–16 Early investigators showed fibrin to be the primary constituent of this thrombus.13,14 Later studies, using refined techniques, clarified morphological and immunologic characteristics of this thrombus. The contribution of fibrin compared to that of platelets in the tumor cell thrombus appears to be less marked, more delayed, and more transient.15,16 In 1971, Jones et al.15 demonstrated the sequence of metastatic events in Sprague-Dawley rats after injection of Walker 256 mammary adenocarcinoma cells. Shortly after injection, these cells were present in the arterioles and capillaries of the lungs. Within 2 hr of injection, platelets were attracted to the area of tumor cells and had partially surrounded these cells (Fig. 1). By 4 hr, platelets had almost completely surrounded the tumor cells. During this time, the tumor cells had firmly attached to the vascular lining and had begun to encroach upon the vascular wall. Shortly thereafter, platelets began to disaggregate, and by 24 hr, had completely dispersed. Within the next several hours, the tumor cells appeared in the perivascular space, and intense proliferation began. Although there was immunologic detection of fibrin, no morphological evidence of fibrin formation was present. This discrepancy may have been due to the presence of fibrin within the platelets, to reversibility of fibrinogen clots, or to the presence of nonpolymerized fibrin. In another study, Sindelar et al.16 demonstrated the importance of platelet activity in pulmonary metastasis after injection of murine fibrosarcoma cells into C57BL/61 mice. Platelet-tumor cell thrombi were demonstrated in some pulmonary capillaries and arterioles as early as 15 min after injection and were most numerous within 3 hr. The thrombi were mostly formed of aggregated platelets and some tumor cells (Fig. 2). In this study, however, platelet aggregation did not appear to be responsible for primary entrapment of the tumor cells. Platelets seemed to stabilize the initial adhesion of tumor cells to the vascular endothelium. Initially, platelets extended short pseudopodia, which facilitated attachment of tumor cells to the vascular surface; at a later stage, platelets extended long pseudopodia. Again, fibrin formation was not seen morphologically.

These studies suggest that platelets may be more important than fibrin in the thrombus that forms after injection of tumor cells. However, the relatively small amount of fibrin in tumor aggregates does not necessarily reflect that it has a minor role in the tumor thrombus, since fibrin turnover may be increased at the tumor sites. The relative contribution of fibrin and of platelets in the tumor cell thrombus has therefore not been clearly demonstrated.

Effect of Tumor Cells on Platelets and Blood Vessels

Thrombocytopenia

A decrease in platelet count after intravenous injection of tumor cells in some experimental animals has
PLATELETS AND TUMOR METASTASIS

Fig. 1. Walker 256 tumor cell (T) within an arteriole 3 hr after intravenous injection. Many platelets (P) surround the tumor cell, which is still intravascular. Higher magnification of area indicated by arrow reveals presence of "activated" platelets showing partial disintegration. A small amount of fibrin (F) is visible. Magnification 4,300x. Reproduced by permission of Journal of the National Cancer Institute.15

been shown. In studies by Gasic et al., injection of some solid murine tumors (T241, dimethylnbenzanthrine-induced fibrosarcoma and B16, melanoma) and one ascites tumor (15091A, anaplastic mammary adenocarcinoma) produced thrombocytopenia of different degrees (50%-70% reduction in platelet count) within 60 min and lasted for 48 hr.17 Hilgard and associates also detected thrombocytopenia after intravenous injection of Walker 256 murine mammary carcinoma tumor cells in rats.18 The decrease in platelet count correlated with the number of tumor cells injected. Studies using radiolabeled platelets demonstrated retention of platelets in the lungs in association with disappearance from the systemic circulation.

These studies show that injection of tumor cells results in thrombocytopenia, presumably due to trapping of tumor cells by platelets. However, these models do not exactly simulate in vivo metastasis. Under in vivo conditions, tumor cells are slowly released into the circulation. In contrast, in models using injection of tumor cells, tumor cells are released in large numbers rapidly into the circulation. It is possible that injection of other cells or particles would also result in thrombocytopenia. Thus, the relevance of thrombocytopenia to injection of cells and to the metastatic process per se is not exactly clear at this time.

Platelet Aggregation

One aspect of the platelet–tumor cell interrelationship is the ability of some tumor cells to directly stimulate platelet activity in vitro.17,19-27 This finding has led to speculation that platelets may be stimulated by tumor cells in vivo and may then enhance tumor growth and spread. However, no evidence for the ability of tumor cells to stimulate platelet aggregability in vivo has been found. Furthermore, not all tumor cells are capable of this activity. Direct platelet aggregation stimulatory activity in 15 of 31 tumor cell lines tested was shown by Gasic and his colleagues.17 Figure 3 shows typical platelet aggregation curves produced by some of the tumor cells. Prior treatment of the platelet-rich plasma with aspirin produced a reduction in the amplitude of aggregation in response to tumor cells and collagen. The degree of platelet proaggrega-
Fig. 2. Tumor cell embolus in a pulmonary capillary. Section shows tumor cells (TC) present in dense platelet (PI) thrombus 30 min after intravenous injection of dissociated fibrosarcoma cell suspension. One of the tumor cells appears to be adherent to the capillary endothelium (En) along a free surface (arrows) at points of slight ruffling of the cell membrane. Scale 1 μm. Magnification 18,000×. Reproduced by permission of Journal of Surgical Research.

Fig. 3. Platelet aggregation by collagen and tumor cells and inhibition with aspirin. Light transmission through platelet-rich plasma was measured continuously after addition of the agents indicated at 0 time. An increase in light transmission reflects platelet aggregation. When platelet-rich plasma was pretreated with buffer, these agents and 13 other tumors produced platelet aggregation. Pretreatment with aspirin (final concentration 5 mM) reduced the degree of aggregation produced by both collagen and tumor cells. Reproduced by permission of International Journal of Cancer.
tory activity was directly related to the degree of thrombocytopenia caused by the same cells upon injection into experimental animals. Pearlstein et al. demonstrated platelet aggregation stimulatory activity of several lines of polyoma-induced PW20 Wistar-Furth rat renal sarcoma cells. Each cell line varied widely in the ability to produce pulmonary metastasis spontaneously after subcutaneous tumor growth. There was a significant correlation between the in vitro platelet proaggregatory activity and in vivo metastatic potential.

Platelet aggregation induced by some tumor cell lines is preceded by a lag period. This lag period results from complement activation in the virally transformed SV 3T3 cells. The material responsible for platelet aggregation stimulation from these cells has been extracted. This extract, obtained after treatment of the tumor cells with urea, is known to stimulate platelet aggregation directly. The platelet-aggregating material derived from some tumor cell membranes binds to the platelets. This binding requires a plasma factor, which is probably a constituent of the alternate complement pathway. The activated platelet-aggregating material requires a second plasma factor in order to stimulate platelet aggregation. This second factor is not fibrinogen, is not dialyzable at 56°C for 30 min, but is precipitable with 50% saturated ammonium sulfate. However, its exact nature is not clear.

Honn and colleagues have demonstrated that cells from B16a tumors induce platelet aggregation, and this aggregation is inhibited by specific cysteine proteinase inhibitors. The cysteine proteinase responsible for platelet aggregation is probably cathepsin-B, since a cathespin-B-mimicking agent, papain, induces platelet aggregation similar to that induced by B16a tumor cells.

Interaction of Platelets With Blood Vessels

Tumor cells, besides affecting platelet activity, also influence platelet–vessel wall interaction. Under normal conditions, platelets traverse the vasculature without attaching to the vessels. Platelet deposition to the normal endothelium is prevented by heparin-like mucopolysaccharides and by prostacyclin synthesis by the normal endothelium. Prostacyclin dilates the vascular lumen and inhibits platelet adhesion and aggregation. However, once the endothelial lining is damaged, the protective mechanisms against platelet thrombus formation are lost, allowing platelets to adhere to subendothelial collagen. Tumor cells that stimulate platelet aggregatory activity are particularly prone to attach to the denuded blood vessels. In studies by Marcum et al., suspensions of platelets and HUT 20 (an undifferentiated murine tumor) cells with in vitro platelet aggregatory activity adhered to the subendothelium of rabbit aortae, whereas other tumor cells without similar in vitro aggregatory activity did not. The adherence of HUT 20 cells was especially prominent when the subendothelium had been treated with chymotrypsin, thereby exposing fibrillar collagen. The adhesion of platelet–tumor cell aggregates was decreased when the vessel was treated with prostaglandin E2 or prostacyclin, both of which have platelet inhibitory actions; however, aspirin did not affect deposition of these platelet tumor cell aggregates, although it reduced the in vitro aggregation stimulatory activity of the tumor cells.

Prostaglandin Generation

Tumor cells also directly affect the prostaglandins that are generated from platelets and the blood vessels. In the vessel wall, arachidonic acid is converted to cyclic endoperoxides, PGG2 and PGH2, and then to prostaglandins of E, D, F, and I series. Prostacyclin or PGI2 is the major prostaglandin produced by the vessel wall and has potent vasodilator and platelet inhibitory actions. In platelets, arachidonic acid is metabolized to labile endoperoxides, and then mainly to thromboxane A2. Thromboxane A2 is the most potent of platelet-generated prostaglandins and is a vasoconstrictor and platelet proaggregant. The interaction between prostacyclin and thromboxane A2 is thought to be largely responsible for thromboresistance. The normal thromboresistance may prevent tumor cells from attaching to platelets and subsequently from adhering to the vascular surface. In support of this concept, experimental work by Honn and associates shows that prostacyclin, given exogenously, or stimulation of endogenous prostacyclin results in decreased incidence of metastasis when mice are injected with B16 amelanotic melanoma cells. In our experience, patients with bone tumors at high risk of developing metastasis have abnormal prostaglandin generation. Patients with bone tumors have extremely low plasma concentrations of 6-keto-PGF1α (stable hydrolysis product of prostacyclin), whereas plasma concentrations of thromboxane B2 (stable metabolite of thromboxane A2) are in the normal range. Arterial tissues from patients with malignant tumors generate only small amounts of prostacyclin compared to similar vessels from individuals without malignancy. Patients with malignant bone tumors also have deficiency of plasma factors responsible for stabilization of prostacyclin. These studies suggest that patients with malignant tumors have decreased production and sta-
bility of prostacyclin. Whether defects in prostacyclin synthesis and stabilization are a cause of tumor metastasis or occur secondary to metastasis is not known.

Effects of Platelets on Tumor Cells

Stimulation of Growth

Platelets have been recognized to exert direct stimulatory action on tumor cells. These actions are mediated through various platelet-release products. Platelet-specific proteins include platelet factor-4, β-thromboglobulin, platelet basic protein, and platelet growth factor. Platelet basic protein shares immunogenic sites with β-thromboglobulin and with low-affinity platelet factor-4. It has strong mitogenic activity, as shown by incorporation of 3H-thymidine in mouse 3T3 fibroblasts cultured in serum-poor medium. Platelet-derived growth factor, identified by Ross and Vogel, is essential for the stimulation of DNA biosynthesis and cell division in confluent populations of various cell lines, including BALB 1C-3T3 cells, skin fibroblasts, chicken fibroblasts, human glial cells, and smooth muscle cells of man and monkey. During the past several years, platelet-derived factors for growth of tumor cells have been identified. A platelet-derived growth factor for SV40 virus-transformed cells has been identified and characterized. This growth factor was shown to reside in the platelet-dense granules. Recently, Cowan and Graham demonstrated that platelet-derived factors induced stimulation of growth of 48 different tumors in culture. In addition, certain platelet-derived growth factors may stimulate in vivo tumor activity. For example, Witoski and associates demonstrated that a platelet extract increased metastatic potential of malignant herpes simplex virus-2-transformed hamster embryo fibroblast cells. When platelets and malignant cells were injected together, hamsters who received tumor cells alone did not develop these tumors. The in vivo significance of these findings is not clear. Platelet-derived factors stimulate many cells and their stimulation of malignant cell growth may therefore not be specific.

Permeability of Tumor Cells Through Vessel Wall

Platelet-derived factors that affect vascular permeability may also affect metastatic potential. For example, certain vasoactive substances may facilitate the passage of tumor cells through the vessel wall. Platelet-derived vascular permeability factor, in particular, may damage the blood vessels and permit cells to pass through. Platelet-derived vascular permeability factor has been characterized by Nachman and colleagues; this factor is a nondialyzable heat-stable human cationic protein with a molecular weight of approximately 30,000 and is located in the intracellular granules.

Effect of Thrombocytopenia on Tumor Metastasis

Whereas platelet activity stimulates growth and spread of tumor cells, thrombocytopenia has the reverse effect. In one of the pioneering studies in this area, Gasic and associates noted that treatment with neuraminidase protected mice against metastasis after injection of TA 3 ascites tumor cells; this protective effect was shown to be due to concurrent thrombocytopenia, rather than to removal of sialic acid from the membrane. Other agents that result in thrombocytopenia, i.e., antiplatelet serum, also resulted in protection against metastasis in their studies. Injection of some of these agents, such as Vibrio cholerae, neuraminidase, and antiplatelet serum, resulted in initial thrombocytopenia followed by thrombocytosis. When tumor cells were injected during the period of thrombocytopenia, resistance to metastasis was identified; whereas when the same cells were injected during the period of thrombocytosis, this protective effect was not evident.

These studies, showing protective effects of thrombocytopenia against metastasis, strengthen the speculation that platelet activity promotes susceptibility to metastasis of certain tumors.

TRIALS OF PLATELET INHIBITORY AGENTS IN SUBJECTS WITH CANCER

During the past several years, trials of platelet inhibitory agents in several experimental animals have been conducted. Many of these studies have provided equivocal results. A major limitation to these studies has been the lack of a good model of tumor metastasis. Injection of tumor cells into the animals is the most frequent way in which metastasis is simulated. However, there are several critical differences between this procedure and normally occurring metastasis in man. In man, a continuous outflow of cells, rather than a simple bolus injection, occurs. The cell number injected into animals is therefore much greater in a given time period than what may be expected in man. In this setting, tumor cell deposition in a specific area may, in effect, represent tumor embolism rather than tumor metastasis. In addition, the cells injected may not represent those that would have metastasized from the primary tumor. Another difficulty relates to the use of allogeneic tumor cells in most cases. The effect of immunologic factors in passing genetic and sometimes even species barriers cannot, therefore, be controlled. Finally, in the presence of a primary neoplasm, there is a modification of several physiologic para-
ters in natural conditions that does not happen in the animal models under simulated conditions.

Nevertheless, there have been several interesting studies examining platelet inhibitory drugs as potential antimetastatic agents. Some of these drug trials are listed in Table 1. The agents used have been cyclooxygenase inhibitors, phosphodiesterase inhibitors, and prostacyclin or prostacyclin-stimulating agents.

Results with cyclooxygenase inhibitor aspirin have been equivocal. Although favorable results were reported by Gasic et al.\textsuperscript{17} and by Kolenich et al.,\textsuperscript{48} others\textsuperscript{18,49} have not detected beneficial effects. There may be individual differences due to the differences in tumor cell and host sensitivities. However, it is now known that aspirin inhibits prostaglandin generation both in the vessel wall and in the platelets. Since prostacyclin and thromboxane A\textsubscript{2} would both be inhibited by aspirin, the net effect on the equilibrium of these two major prostaglandins may be negligible or even detrimental.

Phosphodiesterase inhibitors (pentoxifyllin, RA 233, theophylline) appear to be promising agents. These drugs increase intracellular cyclic adenosine monophosphate (AMP) level and inhibit platelet serotonin release and thromboxane A\textsubscript{2} generation.\textsuperscript{50,51} These agents also potentiate the platelet aggregation inhibitory actions of prostacyclin\textsuperscript{52–54} and increase endogenous prostacyclin production.\textsuperscript{54} The net effect of inhibition of thromboxane A\textsubscript{2} and of increase in production and bioactivity of prostacyclin, therefore, may be promising in the inhibition of metastasis.

Recent reports on the effects of agents acting on specific sites on arachidonic acid metabolism appear very promising.\textsuperscript{34,35,55} Pretreatment with thromboxane-A\textsubscript{2} synthetase inhibitors or thromboxane A\textsubscript{2} receptor antagonist was shown to provide protection against formation of metastasis in mice injected with B16a melanoma cells.\textsuperscript{55} Similarly, pretreatment with prostacyclin or theophylline and prostacyclin together was protective in these mice (Fig. 4).\textsuperscript{34} The mechanisms by which prostacyclin decreases the metastatic potential of some tumor cells may vary. Some investigators have shown that high intracellular cycle AMP levels inhibit cell division and promote differentiation.\textsuperscript{57} The

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<tr>
<th>Agent</th>
<th>Action</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclooxygenase inhibitors</td>
<td>Inhibit platelet aggregation (2nd wave), release, and prostaglandin generation</td>
<td>Aspirin-treated mice injected with MCA2 cells or T241 cells had a decrease in metastasis (42% and 62%, respectively)</td>
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<td>Aspirin-treated mice injected with BW 10232 adenocarcinoma cells had a 50% decrease in metastasis</td>
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</tr>
<tr>
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<td></td>
<td>Aspirin-treated rabbits injected with V\textsubscript{2} carcinoma cells had no change in metastasis</td>
<td>49</td>
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<tr>
<td></td>
<td></td>
<td>Aspirin-treated mice injected with Lewis lung carcinoma had no change in metastasis</td>
<td>18</td>
</tr>
<tr>
<td>Phosphodiesterase inhibitors</td>
<td>Increase cyclic AMP concentrations in platelets; decrease platelet aggregation; and increase prostacyclin generation and activity</td>
<td>Pentoxifyllin-treated rats injected with Wilms tumor cells had 75% reduction in metastasis; injected with neuroblastoma cells had 31% decrease; and injected with adrenocarcinoma cells had no change in metastasis</td>
<td>56</td>
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<td>RA 233-treated mice injected with Lewis lung carcinoma cells had no change in metastasis</td>
<td>18</td>
</tr>
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<td>Theophylline potentiated antimetastatic effects of prostacyclin in mice injected with B16 amelanotic melanoma cells</td>
<td>34</td>
</tr>
<tr>
<td>Prostacyclin and prostacyclin stimulants</td>
<td>Inhibit platelet adhesion and aggregation; vasodilation</td>
<td>Prostacyclin-treated mice had 75% reduction in pulmonary metastasis and 100% reduction in liver metastasis after injection of B16 amelanotic melanoma</td>
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<td>Nafazatrom-treated mice showed similar reduction in metastasis after injection of B16 amelanotic melanoma</td>
<td>35</td>
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increase in cyclic AMP resulting from prostacyclin administration may decrease tumor replication and proliferation. Nafazatrom, which is believed to stimulate endogenous prostacyclin, also exerted antimetastatic effects in other studies.35

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Potential role of platelets in the pathogenesis of tumor metastasis

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