Anticoagulant therapy of cancer patients: Will patient selection increase overall survival?

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Summary
Already since the early 1800s, it has been recognised that malignancies may provoke thromboembolic complications, and indeed cancer patients are at increased risk of developing venous thrombosis. Interestingly, case control studies of deep-vein thrombosis suggested that low-molecular-weight heparin (LMWH) improved survival of cancer patients. This led to the hypothesis that cancer cells might ‘take advantage’ of a hypercoagulable state to more efficiently metastasise. Initial randomised placebo control trials showed that LMWH improve overall survival of cancer patients, especially in those patients with a relatively good prognosis. The failure of recent phase III trials, however, tempers enthusiasm for anticoagulant treatment in cancer patients despite an overwhelming body of literature showing beneficial effects of anticoagulants in preclinical models. Instead of discarding LMWH as potential (co)treatment modality in cancer patients, these disappointing recent trials should guide future preclinical research on anticoagulants in cancer biology. Most and for all, the underlying mechanisms by which coagulation drives tumour progression need to be elucidated. This could ultimately allow selection of cancer patients most likely to benefit from anticoagulant treatment and/or from targeted therapy downstream of coagulation factor signalling.

Keywords
Blood coagulation, heparin, low-molecular-weight, patient selection, receptors, proteinase-activated, neoplasms

Introduction
Venous thrombosis is a well-known complication of cancer that is associated with disease progression and poor prognosis. Anticoagulant treatment is therefore common practice in cancer patients. Intriguingly, anticoagulants are not only postulated to prolong survival by preventing thrombotic complications but anticoagulant treatment may also limit metastasis thereby prolonging survival. However, recent clinical trials do not confirm any survival benefit of anticoagulants independent from preventing thrombosis. The importance of anticoagulants in cancer patients is therefore under debate. Before dismissing anticoagulants as potential anticancer treatment it is important to realise, however, that highly successful targeted therapies for cancer only benefit a small subgroup of patients. Here, we postulate that appropriate patient selection may therefore ultimately reveal the real efficacy of anticoagulants in cancer patients.

In this review, we first discuss the fact that cancer predisposes to thrombotic complications and we will elaborate on the relevance of hypercoagulability in cancer. Subsequently, we discuss the potential clinical importance of anticoagulant therapy for cancer patients including both initial trials showing increased survival of cancer patients on anticoagulant treatment and recent data that shed doubt on the efficacy of anticoagulants in cancer patients. Next, we provide potential explanations for the limited effect of anticoagulants in the recent trials and we suggest that patient selection based on biological characteristics of the tumour may significantly increase the efficacy of anticoagulant treatment in future trials. Finally, we provide several challenging suggestions to select patients that will benefit from anticoagulant treatment, and we will provide future perspectives in the field of coagulation and cancer.

Cancer predisposes to thrombotic complications
The first description of a relationship between cancer and coagulation stems from 1823 when Dr. Bouillaud documented the occurrence of peripheral oedema in the legs of cancer patients. He suggested the oedema to result from obstruction of the veins by fibrin clots that were induced by the cancerous process (1, 2). Forty-two years later, Dr. Trousseau defined the relationship between cancer and venous thromboembolism (VTE) and he named the
syndrome “phlegmasia alba dolens” (acute white and painful inflammation) (3), nowadays better known as “Trousseau’s syndrome” (4). Since these landmark publications, many studies focused on the relationship between VTE and cancer and it now well established that VTE is a frequent and important clinical complication in patients with malignancy. The risk for VTE is approximately 6-7 times higher in cancer patients compared to non-cancer patients (5, 6), but the risk is highly dependent on the cancer type (7, 8), stage of disease and treatment (9).

Relevance of hypercoagulability in cancer

Thrombosis is associated with advanced-stage disease and a poor prognosis in cancer patients. Indeed, in a large Danish study of over 34 thousand patients, distant metastasis was increased in patients in whom cancer was diagnosed within one year after an episode of VTE as compared to cancer patients without VTE (10). Moreover, the one-year survival rate was around three fold reduced in patients with cancer and VTE (10). In line, the cumulative probability of death within six months of hospitalisation is 42% for cancer patients without VTE vs 94% for cancer patients with concurrent VTE (11). Interestingly, hypercoagulability in cancer patients may not just be associated with unfavourable prognosis but actually seems to contribute to disease progression. Preclinical experimental animal models provide intriguing data that cancer cells may exploit coagulation factors to increase their survival and/or dissemination. Indeed, infusion of minute amounts (i.e. 250–500 mU) of thrombin enhances experimental metastasis of both colon cancer and melanoma cells (12). In addition, thrombin augments cancer cell adherence to platelets and thrombin-treated cancer cells metastasize more efficiently in experimental pulmonary metastasis models (13). In line with an important role for thrombin in metastasis, FVLeiden mice with a genetic predisposition for enhanced thrombin generation are prone to develop metastasis, whereas haemophilic FVIII-deficient mice are protected against experimental cancer cell metastasis (14). Moreover, metastasis is enhanced in hypercoagulable mice due to reduced endogenous APC levels (15, 16), whereas metastasis is largely reduced in heterozygous prothrombin-deficient mice (17). Finally, numerous studies show that anticoagulants targeting FXa and/or thrombin limit metastasis and prolong survival in experimental animal models (18–22). For instance, the direct thrombin inhibitor hirudin limits cancer cell extravasation and subsequent metastasis and hirudin prolongs survival of mice implanted with subcutaneous tumours (23). Likewise, novel direct thrombin inhibitors, like dabigatran and argatroban, inhibit breast cancer invasion and metastasis (24–26).

Anticoagulant therapy and cancer patients

From a clinical perspective, the first studies reporting favourable outcomes after anticoagulant treatment of cancer patients dealt with vitamin K antagonists (VKA). Enthusiasms for VKA treatment in cancer patients, however, faded a decade ago due to a systematic literature review indicating that there is no evidence to support VKA therapy for prolonging survival in cancer patients (27). Nevertheless, the use of VKA limits the incidence of certain tumour types such as prostate cancer (28). In contrast, but in line with the prime hypothesis that coagulation contributes to cancer progression, low-molecular-weight heparin (LMWH) may prolong overall survival of cancer patients. Indeed, it was shown that during a six month follow-up period, 44% of cancer patients treated with standard heparin died as opposed to only 7% of cancer patients treated with LMWH (29). As elegantly stated by the authors, such a difference is statistically unusual if LMWH would have no effect on the likelihood of succumbing to cancer. Subsequent meta-analyses of clinical trials aiming at VTE treatment confirmed that LMWHs prolonged overall survival in subgroups of cancer patients (30–32). Importantly, the mortality reduction did not result from differences in death related to VTE and was observed in a variety of cancer patients.

The first randomised, double-blind, placebo-controlled trial to assess the efficacy of LMWH in cancer patients without underlying thrombosis (FAMOUS trial including 385 patients with advanced malignancy) showed promising effects (33). Although the one-year overall survival did not differ between the LMWH and placebo groups (survival rates of 46 vs 41%), subgroup analysis revealed that patients with a better prognosis did benefit from LMWH treatment (Table 1). Indeed, median survival increased from 24% in the placebo group to 44% in the LMWH group. Two subsequent randomised trials confirmed that LMWH could prolong survival of patients with small cell lung cancer (n=84 (34)) or with metastasised or locally advanced solid tumours (n=300 (35)) and especially in patients with a better prognosis at enrollment (35). Unfortunately, however, only a small study including 38 lung cancer patients with limited disease could confirm the survival benefit of LMWH treatment in subsequent studies (36). Several other trials, with an overall inclusion of over 5,000 patients with solid tumours of the pancreas, lung, breast, gastrointestinal tract and brain, did not show any effect of LMWH on overall survival (37–43). Overall, anticoagulants thus seem to have a limited effect in cancer patients, a notion confirmed by two recent meta-analyses either showing that LMWH does not affect the overall survival of cancer patients (44) or that heparin may have a small effect on mortality (risk ratio 0.97 [0.92–1.01] and 0.95 [0.90–1.00] at 12 and 24 months, respectively) (45).

Potential explanations for the limited effect of anticoagulants in cancer patients

Despite an overall belief that anticoagulants may be associated with a survival benefit in cancer patients, the clinical evidence as outlined above remains scarce and unconvincing. Several explanations for the disappointing results of the clinical trials have been put forward. Most and for all, enrolment of patients with end-stage disease may have obscured the overall effect of anticoagulants. Indeed, already in the initial clinical trials, patients with limited
disease, and consequent better prognosis, seemed to benefit most from anticoagulants (33, 35). In line, LMWH treatment did improve survival in patients with solid tumours without metastatic disease at the time of an acute VTE but it did not improve survival in patients with metastatic disease (46). Moreover, two of the trials that included (mainly) patients with limited disease reported the most significant reductions in overall mortality (34, 36), again arguing that anticoagulants may be particularly effective in patients with limited disease. However, excluding patients with limited disease did not decrease the effect of anticoagulant treatment on overall survival in a meta-analysis (47). Whether anticoagulants indeed increase overall survival specifically in patients with limited disease thus remains to be established. The fact that anticoagulants mainly limit metastasis in experimental animal models would argue in favour of such a hypothesis, however.

Interestingly, the majority of the abovementioned clinical trials enrolled patients with a wide variety of primary cancers. It may well be that anticoagulants are not effective against every cancer type. In support of this hypothesis, hirudin inhibited experimental metastasis of B16 melanoma cells to the lungs but did not affect lung colonisation of CT26 colon cancer cells (48). Moreover, prophylactic plasma levels of LMWH did not reduce experimental metastasis of colon cancer cells to the liver (49, 50), whereas hypercoagulability due to the FV Leiden mutation also did not affect metastasis to the liver (51). Clinical trials do, however, not show large differences in response rate of anticoagulants depending on the cancer type arguing against the hypothesis that anticoagulants act in a tumour type-specific manner (38, 40). However, these data should be interpreted with care as these trials included a large proportion of end-stage disease patients with consequent poor prognosis and short life expectancy. As discussed above, anticoagulants may not be effective in these patients.

It is tempting to suggest that anticoagulant treatment strategies are not optimal for limiting metastasis in cancer patients. As suggested before (52), anticoagulant treatment may reduce endogenous APC generation by the thrombin-thrombomodulin complex thereby provoking metastasis (15, 53). This could imply that anticoagulant treatment in cancer patients should be designed to reduce thrombin formation without affecting APC generation. An elegant study shows that long-term thrombin inhibition increased experimental metastasis (54) while in a similar model short-term dosing regimens did reduce metastasis. Properly designed anticoagulant treatment strategies could thus improve its efficacy in cancer patients. However, the different treatment modalities in the clinical trials discussed above do not provide clear evidence to support this notion. It is thus unlikely that alternative treatment strategies will considerably increase the success of anticoagulants for increasing overall survival of cancer patients.

### Anticoagulants and patient selection

Treatment of cancer patients is rapidly changing due to the development of targeted therapies acting upon tumour-specific

<table>
<thead>
<tr>
<th>First author (reference)</th>
<th>Year</th>
<th>Number of patients</th>
<th>Major tumour types</th>
<th>Effect on survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kakkar (33)</td>
<td>2004</td>
<td>385</td>
<td>Breast, Colorectal, Ovarian and Pancreatic</td>
<td>1-, 2- and 3-year survival rates 41, 18 and 12 % (placebo) vs 46, 27 and 21 % (LMWH). Median survival 43.5 (placebo) vs 24.3 (LMWH) months for patients with better prognosis at diagnosis.</td>
</tr>
<tr>
<td>Altinbas (34)</td>
<td>2004</td>
<td>84</td>
<td>Lung</td>
<td>Median survival 8.0 (placebo) vs 13.0 (LMWH) months.</td>
</tr>
<tr>
<td>Klerk (35)</td>
<td>2005</td>
<td>302</td>
<td>Breast, Colorectal, Lung, Liver, Pancreatic and Prostate</td>
<td>Median survival 6.6 (placebo) vs 8.0 (LMWH) months.</td>
</tr>
<tr>
<td>Lecumberri (36)</td>
<td>2013</td>
<td>38</td>
<td>Lung</td>
<td>Median survival 11.3 (placebo) vs 37.1 (LMWH) months.</td>
</tr>
<tr>
<td>Sideras (37)</td>
<td>2006</td>
<td>138</td>
<td>Breast, Colorectal and Lung</td>
<td>Median survival 10.5 (placebo) vs 7.3 (LMWH) months.</td>
</tr>
<tr>
<td>Agnelli (38)</td>
<td>2009</td>
<td>1186</td>
<td>Breast, Gastrointestinal, Lung, Ovarian and Pancreatic</td>
<td>Mortality rate after 120 days 4.3 (placebo) vs 4.2 % (LMWH).</td>
</tr>
<tr>
<td>Perry (39)</td>
<td>2010</td>
<td>186</td>
<td>Glioma</td>
<td>1-year survival rate 54.6 (placebo) vs 52.2 % (LMWH).</td>
</tr>
<tr>
<td>van Doormaal (40)</td>
<td>2011</td>
<td>503</td>
<td>Lung, Pancreatic and Prostate</td>
<td>Median survival 11.9 (placebo) vs 13.1 (LMWH) months.</td>
</tr>
<tr>
<td>Agnelli (41)</td>
<td>2012</td>
<td>3212</td>
<td>Colorectal and Lung</td>
<td>1-year survival rate 55.5 (placebo) vs 56.6 % (LMWH).</td>
</tr>
<tr>
<td>Haas (42)</td>
<td>2012</td>
<td>351 532</td>
<td>Breast, Lung</td>
<td>6-month survival rate of 93.3 (placebo) vs 91.2 % (LMWH). 6-month survival rate of 78.4 (placebo) vs 79.8 % (LMWH).</td>
</tr>
<tr>
<td>Maraveyan (43)</td>
<td>2012</td>
<td>123</td>
<td>Pancreatic</td>
<td>Median survival 9.7 (placebo) vs 8.7 (LMWH) months.</td>
</tr>
</tbody>
</table>
pathways (55). These targeted therapies have led to significant improvement in the treatment of several cancers. Importantly, however, only a (small) selection of patients benefit from these targeted therapies while the large majority of patients are not responsive to these state-of-the-art treatment modalities. For instance, only around 10% of non-small cell lung cancer patients respond to epidermal growth factor receptor (EGFR) kinase inhibitors (56, 57). A similar scenario may hold true for anticoagulants, i.e. only a selected group of patients will show objective response rates and these patients need to be identified. However, clinical trials using anticoagulants for cancer treatment do not select patients based on biological characteristics (except tumour type and stage in some studies). Obviously, selecting patients with the highest likelihood to respond to anticoagulants is not an easy task as response-predicting biomarkers have not yet been characterised.

Patient selection criteria for anticoagulant treatment: thought-provoking suggestions

Preclinical (animal) experiments suggest that individual coagulation factors drive metastasis. Anticoagulants may thus prolong overall survival of cancer patients by preventing coagulation-factor dependent pro-tumourigenic effects. Consequently, anticoagulants are likely to be most effective in patients with an evident hypercoagulable state and the applicability of D-dimer and/or the thrombin generation potential as in/exclusion criteria need to be addressed (Table 2).

Coagulation factors are thought to contribute to tumour progression by activating protease activated receptors (PARs) (58). Indeed, thrombin-driven PAR-1 signalling promotes tumour cell proliferation, adhesion and migration (59), whereas TF-dependent PAR-2 activation also seems to induce adhesion and migration (60). More importantly, PAR-1 expression on tumour cells is associated with disease progression (61–63) and inhibition of PAR-1 limits tumour growth and metastasis in experimental animal models (64, 65). Similarly to PAR-1, PAR-2 expression is associated with tumour characteristics; expression associates with size and histological grade in breast cancer (66) and associates with TNM stage, depth of invasion and metastasis in colorectal cancer (67).

Preference for PAR-1 or PAR-2 targeting after coagulation activation is cancer type-dependent. While PAR-2 appears to be crucial to angiogenic switching in in vivo breast cancer models, thrombin-dependent PAR-1 activation occurs in e.g. melanoma (68, 69). Alternatively, PAR-1 and PAR-2 may also function as one signalling unit in cancer cells; PAR-1 activation in certain cancer cells induces transactivation of PAR-2, leading to PAR-1/PAR2-dependent cell migration (70). Overall, it is tempting to speculate that anticoagulants limit cancer progression by inhibiting PAR signalling. Hence, (high) PAR expression may be a prerequisite for effective anticancer effects of anticoagulants. In support of this tantalising suggestion, both LMWH and hirudin inhibit experimental metastasis of PAR-1 expressing B16F10 melanoma cells but not of PAR-1 negative K1735 melanoma or CT26 colon cancer cells (48).

Both PAR-1 and PAR-2 may exert some of their pro-tumourigenic properties via transactivation of the EGFR pathway. Indeed, proteolytic activation of PAR1 by thrombin induces persistent transactivation of EGFR in breast cancer cells thereby promoting cancer cell growth and invasion (71, 72). Similarly, activation of PAR-1 enhances colon cancer cell proliferation (73) and renal cancer cell migration (74) through EGFR transactivation, whereas PAR-2 activation leads to transactivation of EGFR in gastric cancer cells thereby promoting proliferation (75). PAR-2 activation may also lead to crosstalk with the insulin-like growth factor receptor (IGFR) in breast cancer cells (76). Although preclinical data confirming the importance of PAR-dependent EGFR and IGFR transactivation in cancer progression are still lacking, these data imply that anticoagulants may not be effective in patients with activating mutations in these receptors (Figure 1). In addition, it is well-known that EGFR inhibitors lack efficacy in patients with K-RAS mutant tumours (77, 78). Coagulation-PAR-dependent EGFR transactivation may thus be irrelevant for progression of tumours harbouring KRAS mutations suggesting anticoagulants will not have clinical efficacy in patients with KRAS mutant tumours.

Next to PAR signalling, coagulation may also potentiate metastasis by protecting cancer cells from innate immune surveillance early after the establishment of metastatic foci. Activated platelets, together with fibrin, form aggregates in which cancer cells “hide” from natural killer cells thereby enhancing metastasis (79–81). Alternatively, platelet-derived factors may induce tumour cell differentiation making them less vulnerable for natural killer cells (17). Interestingly, platelet-derived transforming growth factor (TGFβ) activates the TGFβ/Smad and NF-κB pathways in cancer cells, resulting in their transition to an invasive mesenchymal-like phenotype leading to enhanced metastasis (82). Anticoagulants may thus limit platelet activation and subsequent TGFβ release thereby preventing tumour cell differentiation and metastasis. Importantly, the TGFβ receptor 2 (TGFBR2) is often mutated in cancer cells frequently leading to inactivation of the TGFβ pathway (83). Such a scenario would directly suggest that patients with tumours harboring inactivating TGFBR2 or SMAD2/4 mutations may not respond to anticoagulant treatment.

An additional and intriguing role for coagulation and concomitant platelet activation in metastasis was recently demonstrated by Gil-Bernabe et al. (84). Tumour cell-dependent coagulation activation in vivo induced localisation of CD11b+ macrophages to the pre-metastatic niche. In parallel, thrombin-activated platelets

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Table 2: Patient selection criteria to increase efficacy of anticoagulants in cancer patients.

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<thead>
<tr>
<th>Exclusion criteria</th>
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<tr>
<td>1. Low D-dimer and/or thrombin generation potential</td>
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<tr>
<td>2. Low PAR expression levels</td>
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<tr>
<td>3. Activating mutations in EGFR and/or IGFR</td>
</tr>
<tr>
<td>4. KRAS mutations</td>
</tr>
<tr>
<td>5. Inactivating mutations in TGFBR2 and/or SMAD2/4</td>
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bound to metastatic tumour cells at the metastatic niche to facilitate recruitment of these macrophages to tumour cell/platelet aggregates. This led to tumour cell survival that was independent of NK cell function. Taken together, coagulation activation leads to several independent events, both NK dependent and independent, in the promotion of metastasis.

**Conclusion and future perspectives**

Cancer induces a hypercoagulable state which (in preclinical animal models) contributes to tumour progression mainly by potentiating metastasis. Anticoagulant treatment may thus limit cancer progression and prolong the life expectancy of cancer patients. Although several clinical trials indeed show increased overall survival of cancer patients treated with LMWH, enthusiasm for anticoagulants in cancer treatment seems to fade due to recent trials that do not confirm the survival benefit of LMWH. Importantly, however, none of the trials selected patients on their likelihood to benefit from anticoagulant treatment, and some of the best treatment options available today would not be available without appropriate patient selection (for instance EGFR and/or HER2 inhibitors). It is consequently pivotal to identify patients eligible for anticoagulant treatment, and to exclude patients that will not respond to anticoagulants. The latter seems particularly important as anticoagulants may induce bleeding complications thereby even decreasing life expectancy of non-responders.

Response-predicting biomarkers are not yet characterised for anticoagulants, and patient selection is thus a challenging task. To allow appropriate patient selection in future studies, basic research need to focus on the underlying mechanisms by which coagulation factors drive cancer progression. Additionally, potential...
bimarkers should retrospectively be analysed in both responders and non-responders in any of the previous anticoagulation trials. In the current review, we highlighted several potential biomarkers like mutations in KRAS, EGFR and TGFBR2 but future studies should elucidate if indeed these are the best biomarkers available to select patients for anticoagulant treatment.

Conflicts of interest
None declared.

References

Thrombosis and Haemostasis 114.3/2015

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