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Optimising Translation of Pleiotropic Research into Clinical Applications: Low Molecular Weight Heparin Treatment in Cancer

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ABSTARCT

The concept of pleiotropy is derived from genetics whereby a locus can influence multiple traits. Applied to pharmacology, pleiotropic effects may be regarded as the multiple influences that a molecule or group of molecules have on biological systems. One class of molecules with an extensive but disparate range of publications over the last forty years reporting clinically relevant pleiotropic effects are the Low Molecular Weight Heparins (LMWH). As clinical practice with regards to use of LMWH in thromboprophylaxis changes and other anticoagulants are adopted, there is a risk of this pleiotropic research becoming obsolete and the full clinical potential of LMWH failing to be realised. To avoid this, the efficient and timely translation of pleiotropic research into clinical applications is vital so patients may benefit from these novel therapeutic avenues of agents whose safety and toxicity profiles are well defined *via* many years of robust clinical evidence.

To overcome this 'translation barrier' a number of carefully designed clinical trials are underway, informed by the new science, with endpoints designed to demonstrate the clinical value of LMWH beyond their licensed anticoagulant effects. Results from earlier studies indicate that whereas some pleiotropic effects are a class effect of LMWHs, others are influenced by the specific LMWH molecule. In cancer, for example, LMWHs have anti-metastatic, anti-angiogenic and immune modulatory effects, but the potency varies by molecule. A recent study in pancreatic cancer, based on current scientific rationale and involving a particular LMWH–tinzaparin - demonstrated an improvement in Progression-Free Survival (PFS); follow-up studies are planned.

To translate researched pleiotropic effects of LMWH into clinical practice, a robust methodology is required to filter, prioritise, and clinically study those that are likely to have clinical impact, such as measurable improvements in progression free survival (PFS) and overall survival (OS) of cancer patients receiving LMWH. For pleiotropic research to be translated into clinical care, this methodology would need to overcome key challenges such as heterogeneity of patients and lack of reliable biomarkers to guide patient selection. This paper outlines the findings from a recently convened scientific exchange panel to review pleiotropic effects of LMWHs on cancer outcomes, and the lessons for pleiotropic research translation into real world clinical practice, culminating in the drafting of a 'Pleiotropic-to-Practice' (P2P) method.

Keywords: Pleiotropy; Pharmacology; Heparin treatment; Cancer

INTRODUCTION

The concept of pleiotropy is derived from genetics whereby a locus can influence multiple traits. Applied to pharmacology, pleiotropic effects may be regarded as the multiple influences that a molecule or group of molecules have on biological systems. In some instances, this has the potential to enhance clinical efficacy and enable a more personalized approach to prescribing. However, a key challenge is translating science into real world clinical applications, where pleiotropic effects are often outside the original licensed indications for marketed medicines.

One such class of molecules with a growing area of pleiotropic research is low molecular weight heparins (LMWH) with over forty years of published pre-clinical and clinical research. This field of research has experienced significant challenges in applying findings to clinical practice, with one consequence being that as clinical prescribing practices evolve with new more targeted agents; the potential benefit of pleiotropic research is at risk of becoming obsolete. For example, over the last ten years, many clinicians have moved away from prescribing LMWH and towards prescribing Direct Oral Anticoagulants (DOACs) for the prevention and therapy of venous thromboembolism. DOACs are highly specific, targeted agents, which while efficacious for thromboprophylaxis may not confer the same pleiotropic anti-cancer effects as has been described for LMWH. The adoption of DOACs means that efficient and timely translation of pleiotropic LMWH research into clinical applications is vital so as not to lose the value of that research [1].

MATERIALS AND METHODS

A recently convened scientific exchange working group brought together key researchers and clinicians studying the pleiotropic effects of LMWHs in cancer progression and survival. Extensive pre-clinical research indicates LMWHs could be associated with a range of beneficial effects including reduction in tumour metastasis and disease progression, along with an improvement in Progression Free Survival (PFS) and Overall Survival (OS) of cancer patients. Work is underway to characterize these effects, building on the plethora of *in vitro* and *in vivo* data that has accumulated over the years.

The emergence of new research technologies has allowed the development of more-focused LMWH clinical trials which are now beginning to yield clinically relevant information. Translating this information into clinically relevant knowledge requires a robust 'integrated' LMWH pleiotropic pathway that promotes collaboration between academic and clinical communities with less siloed working, allowing more efficient profiling and prioritisation of translation 'candidate' effects, and enabling rapid and effective study design and deployment to test newly devised evidence-based hypotheses in the clinic, with a focus on patients stratified and/or selected for optimal benefit.

Prioritization of specific LMWH pleiotropic effects will require a set of 'translation criteria' to identify the right candidates. In the field of cancer, such criteria would indicate a focus on those effects where there is greatest clinical need; where there is most evidence indicating pleiotropic effect for the class or individual LMWH, and where treatment pathways already exist that would allow for rapid and widespread adoption of a new approach to the use of LMWH. Consideration must also be given to the unresolved question of whether pleiotropic effects of LMWH observed in cancer are a class effect, or whether the characteristics of the individual therapeutic heparin preparations confer specific effects.

RESULTS AND DISCUSSION

This report describes the proceedings of a scientific exchange meeting addressing the pleiotropic effects of LMWH, with a particular focus on tinzaparin. The group reviewed the molecular mechanisms by which LMWH in general and tinzaparin in particular exert a pleiotropic effect in cancer; the evidence generation that must take place to demonstrate the pleiotropic effects of tinzaparin in cancer; and the characteristics of pleiotropic effects that are likely to make translation to clinical application feasible. Following this review, a three-stage method was devised to guide translation of pleiotropic effects into clinically meaningful outcomes. A summary of these stages is presented in the following sections.

Stage 1: Review and profiling of LMWH pleiotropic effects research in cancer

LMWH is a class of anticoagulant medication used in the treatment of myocardial infarction and in the prevention and treatment of venous thromboembolism (deep vein thrombosis and pulmonary embolism) including Cancer-Associated Thrombosis (CAT). Heparin is a naturally occurring polysaccharide that inhibits coagulation by accelerating the rate of inactivation of specific coagulation factors by antithrombin. Natural heparin consists of molecular chains of varying lengths, or molecular weights. Heparin chains from 5,000 to over 40,000 Da make up polydisperse pharmaceutical-grade heparin. LMWHs, in contrast, consist of only short chains of polysaccharide. LMWHs are defined as heparin salts having an average molecular weight of less than 8,000 Da and for which at least 60% of all chains have a molecular weight less than 8,000 Da. These are obtained by various methods of fractionation or depolymerisation of polymeric heparin [2].

Tinzaparin is a LMWH indicated for the treatment of venous thrombosis and thromboembolic disease-including deep vein thrombosis and pulmonary embolus in adults and for extended treatment of venous thromboembolism

and prevention of recurrences in adult patients with active cancer. A scan of pleiotropic research literature indicates an active role for tinzaparin in many other (non-antithrombotic) processes through a myriad of interactions, with many of these functions yet to be fully characterised. A proportion of these functions are shared with other molecules in the LMWH class, exerting pleiotropic effects in cancer through immune modulation, tumour cell adhesion and inhibition, heparanase inhibition, and angiogenic growth factor inhibition [3].

More specifically, the molecular mechanisms by which tinzaparin exerts pleiotropic effects in cancer have been suggested to be manifested through anti metastatic rather than anti proliferative properties on primary tumour growth; this is consistent with *in vitro* models, where LMWHs as a class do not appear to significantly attenuate tumour growth. The biological mechanisms responsible for these anticancer properties appear to be multifactorial. In the first instance they may be related to the need for tumour cells to bind to and migrate across vessel walls. Evidence demonstrates that LMWHs can interfere with the interaction between tumour cells and endothelial cells and thus may reduce transendothelial tumour migration. Inhibition of Vascular Endothelial Growth Factor (VEG F) by LMWHs has been reported to limit the proliferation of tumours. LMWH inhibition of tumour angiogenesis likely contributes to restricted tumour access to oxygen with consequences for growth restriction. LMWH indirectly reduces tumour cell growth and tumour cell migration by the induction of TFPI release from endothelial cells. TFPI is the most potent natural inhibitor of tissue-factor induced effects including PAR2-mediated mitogenic effects on tumour cells. This is especially relevant for LMWH with higher molecular weight such as tinzaparin [4]. Blockade of thrombin and P selectin by LMWH inhibits the activation of platelets and prevents tumour cells from using activated platelets as a 'cloak' to evade the immune system [5-7].

There are also 'in-license' pleiotropic effects, which may elucidate new evidence around drug mechanisms of action linked to specific diseases. For example, the ATICKS study (AntiThrombotics Impact on Clot Kinetics and Structure) examined the *in vitro* effects of DOACs (rivaroxaban and dabigatran) and LMWH (tinzaparin) on clotting using thromboelastometry. The study found that tinzaparin had greater clotting efficacy compared with DOACs. Whereas DOACs only reduced time to clot formation, tinzaparin additionally reduced clot velocity and clot firmness. This 'extended action' may be significant in some clinical settings, particularly cancer, which shares many of the pathways found in coagulation and inflammation [8]. Studies are also underway to evaluate the effect of LMWH versus rivaroxaban in the acute phase of Venous Thromboembolism (VTE) management, with a focus on potential anti-inflammatory properties of tinzaparin.

Whilst much of this theoretical knowledge around pleiotropic effects of LMWH has not been translated into clinical practice, the findings are yielding information about potential biomarkers that could be used to match research outputs with clinical studies to evaluate the clinical value of molecules such as tinzaparin with different cancer types and specific patient populations.

Stage 2: Extrapolating insights from pleiotropic research into clinical practice translation rules

Deriving value from this field of research can mean translating micro-level pre-clinical findings to a macro level clinical setting shifting observations *in vitro* to applications *in vivo*. Achieving this means identifying criteria for likely translatability, which can be applied to prioritizing and translating micro-level findings.

One important issue to address is consistency. Although there is an increasingly informed scientific rationale underpinning the pleiotropic effects of LMWH in cancer, a number of pre-clinical studies have generated negative or contradictory findings making candidate effects for translation initiatives difficult to identify. One explanation is that the anti-cancer properties of LMWH were not demonstrated due to earlier research lacking a modern understanding of study design. Studies have varied markedly in LMWH doses, initiation of LMWH treatment and frequency, and

pre-clinical tumors models–all of which are likely to contribute to conflicting findings. This emphasizes the need to identify a method to stratify/select specific cancer sub-types and engage in cross-collaborative research to identify consistent and robust anti-cancer properties that can be translated for patient cohorts.

Despite the many negative or contradictory findings, more recent clinical studies have found positive (non-antithrombotic) effects of LMWH in cancer patients. As an example, a meta-analysis investigating the comparative effects of LMWH, Unfractionated Heparin (UFH) and warfarin on one year survival in cancer patients found that LMWH conferred a greater survival benefit, with a relative risk of 0.88 (p=0.015) [9].A recent study in pancreatic cancer, based on current scientific rationale, demonstrated an improvement in PFS with LMWH treatment, with further studies planned [10].

From this review a number of 'translation rules' can be derived that are more likely to generate successful translation of pleiotropic research into clinical practice. An important first rule in future pleiotropic research is to focus on areas with unmet clinical need, for example cancers in which immunotherapy have limited effect, such as glioblastomas. Other priority areas of clinical need include LMWH synergies with other classes of anticancer treatments. In the case of LMWH medicines and cancer, a priority would be to focus on areas where clinical evidence already exists–for example, LMWH research indicates positive anticancer effects in multiple myeloma, ovarian cancer, pancreatic cancer, and small cell lung cancer. Another important translation rule is to prioritise areas with pre-existing clinical prescribing practice which facilitates recruitment and retention of study subjects; institutions where thromboprophylaxis with LMWH for cancer is already recommended are likely to be more viable study sites for clinical application studies. Cancer types that fall into this category include those with a high burden of cancer-associated thrombosis that would benefit from thromboprophylaxis, such as upper gastrointestinal, genitourinary, and gynaecological cancers.

Evaluation of LMWH in studies designed to demonstrate an improvement in Progression Free Survival (PFS) and/or Overall Survival (OS) have featured heterogeneity of patients and a significant number of factors that influence such outcomes. This implies a further translation rule: that only careful patient selection will allow detection of significant clinical effects and permit intra-class differences to emerge, such as between different LMWH molecules. This is one area where new pre-clinical research could play an important role: matching individual LMWHs to tumour targets, providing data indicating that some LMWHs might be better suited to some cancer types than others. Equally important are sub group analyses of such clinical studies to analyse how individual patient characteristics and between-patient variability might affect LMWH metabolism and patient outcomes.

Moving these insights 'upstream' to foundation-level pleiotropic research indicates the importance of identifying biomarkers that are associated with greater translatability into real-world clinical applications. For example, research around tinzaparin indicates anti-cancer benefits in people with certain cancer mutation biomarkers associated with thrombotic risk, based on scientific understanding of the interplay between coagulation, inflammation, and cancer. Known biomarkers such as tissue factor and P selectin could be used to target patients who might benefit most from the anticancer properties of tinzaparin, leading to a more personalised treatment model. This leads to a further translation rule that alternative study objectives may be more appropriate, such as moving away from current end points such as Progression Free Survival (PFS) and Overall Survival (OS) in favour of smaller, smarter objectives that might predict a PFS advantage. The ultimate goal here is to develop an *in vitro* screening assay, where possible, to quantify the anti-cancer properties of molecules and molecule classes against different tumour types.

Practical considerations such as pharmacodynamics and pharmacokinetics are also important. For example, achieving correct 'pleiotropic dosing' levels for a medicine is critical if translation into clinical effects is to be observed. Pleiotropic translation studies with tinzaparin as an anti-cancer agent can involve administration of relatively high doses of the drug. This in turn generates potential methodological issues including study placebo arms being regarded as potentially unethical, such as in pancreatic cancer where LMWH is highly recommended as thromboprophylaxis.

Conversely, there are clinical scenarios where LMWH is not recommended, and caution is advised where clinical usage and experience is minimal. For example, the use of LMWH at high doses in cancers where thrombosis is rare and thromboprophylaxis is not common, such as breast cancer, potentially results in an unfavourable benefit-to-risk profile due to increased risk of bleeding. Concern around complications, such as bleeding in LMWH usage, can instil reluctance in clinicians and patients to participate in such a study.

This implies another important rule that early engagement of stakeholders such as clinicians and patient advocates in designing pleiotropic-derived clinical studies is key to optimising outcomes. Optimising translation also includes modes of communication with 'pleiotropic stakeholders' including clinicians and patient advocates. Using tinzaparin as an example, pleiotropic stakeholders would include those linked to the acute phase of cancer associated thrombosis management, typically medical oncologists, and those responsible for the longer-term management, typically General Practitioners (GPs). However, in communicating pleiotropic research there must be a clear distinction between 'pre-clinical messages' and 'clinical messages' that will be derived from existing clinical evidence and practice.

Effective communication of pleiotropic research therefore requires careful articulation of messages and language. Again using tinzaparin as an example, the important communication elements would include: i) emphasising the unmet medical need and the opportunity to improve care, ii) relating to patients for whom tinzaparin is licensed (even if the dose of tinzaparin needed to achieve anti-cancer effects in those patients is not licensed), iii) communicating the beneficial results of yet-to-be-planned carefully conducted, randomized clinical studies, iv) addressing the reasons why earlier research with tinzaparin into pleiotropic effects produced mixed results and why we are better placed today to introduce effective anti-cancer tinzaparin treatment strategies, v) conveying risks of losing the benefits of tinzaparin if a patient is switched to a Direct Oral Anticoagulant (DOAC), vi) reassuring clinicians that long term use of LMWH is already practiced in some cancer patients (for example, in melanoma), vii) addressing the risk of bleeding, particularly in patients receiving tinzaparin at high doses, viii) being explicit about the responsibility of the clinician to take proactive action to fulfil their responsibility to their patients, ix) advising adoption of tinzaparin in national and local treatment pathways, and x) differentiating these messages from those about DOACs being directed toward clinicians, particularly GPs.

Regarding study design, ethical and cost barriers make the design and execution of prospective randomised controlled clinical studies difficult. As an alternative researchers should consider the use of real-world data analysis as a viable alternative, such as the retrospective analysis of cancer patient care records, comparing patients receiving LMWH with those receiving other antithrombotic agents such as Direct Oral Anticoagulants (DOACs) and Unfractionated Heparin (UHF). Such an analysis could yield valuable information about differences in the differential anticancer potential of available antithrombotic agents. However, those retrospective comparisons can only be considered hypothesis-generating, with special emphasis to selected subgroups. Those hypotheses will hopefully lead to carefully designed randomized trials with positive results.

Stage 3: Optimising pleiotropic effect translation to clinical applications–Towards a 'Pleiotropic-to-Practice' (P2P) method

A review of pre-clinical and clinical research investigating the pleiotropic effects of LMWH indicates that some if not all these molecules exhibit potential multiple modes of action with antithrombotic action being only one of a potential range of anticancer effects. Realizing the potential clinical benefits of this research means a greater discipline in translating findings into actual clinical applications and practice. In turn achieving this requires working to a set of translation rules. Through this review important translation rules can be posited and consolidated into a 'Pleiotropic-to-Practice' (P2P) method which the authors believe can inform researchers and clinicians on how to collaborate more effectively to drive translation and thereby improve patient outcomes (Table 1).

Table 1. Proposed P2P method for optimizing translation of pleiotropic research into clinical applications.

S.No	Pleiotropic-to-Practice' (P2P) method
1	Focus on areas with an unmet clinical need
2	Focus on areas where clinical evidence already exists
3	Prioritise areas with pre-existing clinical prescribing practice
4	Pay careful attention to patient selection
5	Prioritise identification of biomarkers
6	Consider alternative study objectives that might predict a PFS advantage
7	Where possible develop an *in vitro* screening assay
8	Focus on achieving correct 'pleotropic dosing' levels
9	Be cautious where clinical usage and experience is minimal
10	Prioritise early engagement of stakeholders
11	Be careful with the articulation of messages and language
12	Consider the use of real-world data analysis as hypothesis-generating tool for careful planning of RCTs

CONCLUSION

Important translation principles can be derived from the literature describing pleiotropic research in the field of LMWHs and cancer. These principles can inform researchers and clinicians on how to collaborate and focus most effectively in order to optimise eventual patient benefits. The authors hope that the proposed 'Pleiotropic-to-Practice' (P2P) method can accelerate current and future pleiotropic research not only in the field of LMWHs and cancer, but also in a wide range of other diseases. For this to occur requires leadership, with researchers and clinicians taking responsibility for the application of the P2P method. Through this approach, perhaps more of the extensive effort and investment in pleiotropic research can be translated into actual clinical applications with consequent patient benefit.

COMPETING INTERESTS STATEMENT

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