Commonly prescribed drugs and association with breast, colorectal and lung cancer progression: a nested case-control study

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1.0 Background:

There is increasing evidence that commonly prescribed cardiovascular medications such as beta-blockers, angiotensin converting enzyme inhibitors and angiotensin II type I receptor blockers, bisphosphonates (drugs used to prevent fractures in the elderly) and analgesics such as non-steroidal anti-inflammatory drugs and aspirin, may have unintended beneficial effects in relation to cancer prevention and or progression [1]. The following paragraphs outline the pleomorphic properties of these drug classes with respect to cancer progression and collate the epidemiological evidence from studies of their use in lung, colorectal and breast cancer patients. Given the widespread (and generally safe) use of these drugs, their potential for use in the management of cancer warrants further investigation.

1.1 Non steroidal anti-inflammatory drugs (NSAIDs) and cancer progression

NSAIDs are commonly prescribed analgesics for the relief of pyrexia, musculoskeletal pain and inflammation but in recent years long-term use of both cyclooxygenase-2 (COX-2) non selective inhibitors (i.e.: aspirin and ibuprofen) and selective COX-2 inhibitors (i.e.: celecoxib) has been shown to reduce the risk of several cancers including colorectal, breast, lung, pancreatic, oesophageal and prostate cancer[2, 3]. COX inhibition is hypothesized as one mechanism through which NDSAIDs may reduce cancer risk. COX is an enzyme important in the formation of prostaglandins, prostacyclin and thromboxane and pharmacological inhibition of prostaglandin synthesis has been shown to inhibit tumour proliferation [4]. Other anti-cancer effects of NSAIDs includes the promotion of apoptosis, reduction in angiogenesis through reduced vascular endothelial cell growth factor (VEGF) expression and NSAID induced inhibition of tumour metastasis [5].

The majority of studies thus far have examined aspirin and NSAIDs in relation to cancer incidence and provide consistent evidence of decreased incidence of several cancers, especially colorectal cancer, in NSAID users. Few studies have examined NSAID use and cancer progression. There have only been three observational studies to date that have examined the effect of NSAIDs on breast cancer mortality and progression. Blair *et al* [6] observed a reduction in all-cause and BC specific mortality amongst women self-reporting any versus no use of NSAIDs HR 0.57 (95%CI 0.40-0.81) and HR 0.64 (95%CI 0.39-1.05) respectfully. In a further prospective study of 2292 early-stage breast cancer survivors [7], the authors found a significant reduced risk of BC recurrence with ibuprofen RR 0.56 (95%CI 0.32, 0.98) but not aspirin. In a more recent prospective cohort study of 4164 female nurses with early stage (I, II or III) BC, Holmes *et al* [8] found that the risk of BC death decreased as

the frequency of aspirin use increased with relative risks ranging from 0.29 – 1.07. Similar results were observed for distant BC recurrence, with daily aspirin use according a RR of 0.57 (95%CI 0.39, 0.82). Again however information on NSAID usage was self-reported in this study and no information was provided regarding the dose of aspirin taken. More robust evidence on BC mortality and cancer progression among early stage BC survivors may be obtained through observational studies that use prescribing records.

Both aspirin and non-aspirin NSAIDs have been shown to lower the risk of CRC, and recent evidence suggests that NSAID use in patients with colorectal cancer improves cancer-specific and overall survival, particularly in patients with tumours that express COX-2 [9]. Several studies have investigated the effects of NSAIDs in relation to clinical outcomes after CRC diagnosis. Zell *et al* [10] examined pre-diagnostic NSAID use in relation to CRC mortality and found that any (self-reported) NSAID use vs. none was associated with improved overall survival HR 0.71 (95%CI 0.53 – 0.95) and CRC specific mortality HR 0.58 (95%CI 0.40, 0.84). Din *et al* [11], in a large Scottish population based case-control study found that pre-diagnostic use of low-dose aspirin (>4 days/wk for 1 month of more) was not associated with all-cause or CRC-specific mortality. Recently however Rothwell *et al* [12] examined deaths due to cancer during and after RCTs of daily aspirin vs. control in eight trials originally established for the prevention of vascular events and found that allocation to aspirin reduced all cancer mortality by 34%. In a sub-analysis of cancer site, daily aspirin use was associated with a 22% reduction in CRC-specific mortality with this risk improving to a 59% reduction with increased duration of treatment. Analysis on longer follow-up durations i.e.: 10-20 years of follow-up time (179 CRC deaths) demonstrated marked increases in CRC survival HR 0.51 (95%CI 0.35, 0.74; pvalue <0.001). In the same study LC specific mortality was reduced by 29% HR 0.71 (95% 0.58, 0.89; pvalue 0.002), an analysis based on 326 LC deaths. Fuchs *et al* [13] prospectively studied 830 patients with stage III CRC enrolled in a randomised trial of post-operative adjuvant chemotherapy. Consistent aspirin users vs. non aspirin users had a HR of 0.45 (95%CI 0.21, 0.97) for disease recurrence, a HR of 0.48 (95%CI 0.24, 0.99) for disease recurrence/death and a HR of 0.52 (95%CI 0.19, 1.46) for CRC death. Midgley *et al* [14] conducted a phase III randomised trial assessing rofecoxib in the adjuvant setting of CRC. Unfortunately the study was affected by the worldwide withdrawal of rofecoxib, however some 1167 patients received the intervention and a further 1160 received placebo (mean follow-up 4.85 yrs), there was no difference in all cause or CRC specific mortality between the two arms and COX-2 expression was not associated with poorer prognostic or predictive effects. Further studies of the association between use of NSAIDs and cancer progression are warranted.

1.2 Bisphosphonates and cancer progression

Bisphosphonates are the pharmacologic treatment of choice for preventing reduced bone mineral density (BMD) and fractures amongst postmenopausal women [15] and are therefore commonly prescribed in the treatment of osteoporosis/osteopenia [16, 17], but they have additional use in the treatment of hypercalcemia and the prevention of bone metastasis and other conditions involving bone fragility [15, 18, 19].

The potential anticancer effects of first generation bisphosphonates such as clodronate i.e.: non-nitrogen containing bisphosphonates were reported by Powles *et al* [20] who found a significant reduction in the occurrence of bone metastasis amongst those in receipt of clodronate HR 0.44 (95%CI 0.22, 0.86). Interestingly the authors also noted a significant reduction of non-osseous metastasis in the treatment arm. In a subsequent survival analysis of the same participants [21] with a mean of 5.6 years of follow-up, the authors confirmed that oral clodronate significantly improved the five year bone relapse free survival in all patients over the five year study period HR 0.69 (p=0.04) with differences most pronounced in patients with stage II/III disease. In a similar prospective randomised controlled study Diel *et al* [22], reported a significant improvement in overall survival in the clodronate group with 20.4% of patients in the intervention arm dying in the 8.5 years of follow-up vs. 40.7% of control patients. In contrast other trials have found no clinical benefit of clodronate in terms of metastasis prevention or an improvement in survival [23].

In recent years a variety of preclinical and clinical studies have illustrated that the action of nitrogen containing bisphosphonates (alendronate, risedronate, ibandronate, pamidronate, and zoledronic acid) go beyond preventing osteoclast-mediated bone resorption and have demonstrated anti-cancer activity. For instance in vitro studies have shown that bisphosphonates inhibit tumour cell adhesion and invasion, induce tumour cell apoptosis, reduce tumour cell viability and proliferation and exhibit anti-angiogenic effects [24]. There is also recent evidence to suggest that there is a synergistic anti-cancer effect of adjuvant bisphosphonate in combination with chemotherapeutic agents in breast cancer patients [25]. Emerging evidence from RCTs suggests that both oral and intravenous nitrogen containing bisphosphonates may reduce breast cancer recurrence. For instance Brufsky *et al* [26] in an interim analysis of 1,667 women from two on-going trials, Z-FAST and ZO-FAST (Zometa-Femara Adjuvant Synergy Trial), found that the group of women who had received zoledronic acid concurrently with letrozole had a significantly lower rate of cancer recurrence. In a further analysis of 36 months of follow-up from this trial, Eidhmann *et al* [27] reported a 41% reduction in disease-free survival events amongst those receiving zoledronic acid plus adjuvant letrozole compared to those receiving letrozole alone HR 0.59 (95%CI 0.36, 0.96). Gnant *et al* [28] in

an RCT of 1803 premenopausal women with endocrine responsive early stage breast cancer, showed that the addition of a bisphosphonate to the endocrine therapy vs. endocrine therapy alone resulted in a 36% reduction in the risk of disease progression HR 0.64 (95%CI 0.46, 0.91) but did not impact on overall mortality HR 0.60 (95%CI 0.32, 1.11). In both studies the addition of bisphosphonate reduced disease recurrence in both bone and non-bone sites such as the contralateral breast; an effect which has been hypothesised to arise from bisphosphonates beneficial effect on the bone marrow microenvironment in which dormant tumour stem cells would normally survive in early stage disease [29].

In a recent meta-analysis, Mauri *et al* [30], have shown that adjuvant use of bisphosphonates was not associated with overall mortality OR 0.71 (95%CI 0.48-1.04) or BC recurrence OR 0.84 (95%CI 0.60-1.18); however significant heterogeneity was observed in both analyses. In sub group analysis the authors found that zoledronic acid was associated with a 32% lower risk of disease recurrence. Of note the results of this meta-analysis highlighted a non-significant trend towards better outcomes (fewer bone metastases, deaths and local/distal recurrences) amongst bisphosphonate users vs. non users. This latter finding is in agreement with Rennert *et al* [31] who have reported that women receiving bisphosphonates who developed breast cancer had tumours with better prognostic features, including a lower proportion of human epidermal growth factor receptor – 2 (HER2) positive tumours, compared with women who did not receive bisphosphonates.

Taken as a whole, there is mounting evidence that bisphosphonate therapy for postmenopausal osteoporosis might significantly reduce the risk of breast cancer progression and also aid in the prevention of breast cancer recurrence in women with early-stage breast cancer, however other cancers have infrequently been examined.

1.3 Beta-Blockers and cancer progression

Beta-blockers are a class of drugs which are particularly useful for the management of cardiac arrhythmias, cardioprophylaxis following myocardial infarction and cerebrovascular events and hypertension [32, 33]. Noradrenaline (norepinephrine) and adrenaline (epinephrine), the major neuroendocrine transmitters of the sympathetic nervous system 'fight or flight' response, bind to and activate adrenergic receptors or adrenoreceptors [34]. Beta-2 adrenergic receptors (β_2AR) have been shown to be present on pancreatic, breast and ovarian cancer cells [35] leading some to posit that norepinephrine may be an aetiological factor in various types of cancer [36]. In addition, in vitro cell line studies of colorectal [37], prostate [38] and breast cancer [39] have shown stimulation of β_2AR via the stress catecholamine hormone norepinephrine to be a potent inducer of cell migration,

a prerequisite to metastasis formation; highlighting a role of norepinephrine in cancer progression. Moreover β_2AR stimulation has been associated with resistance to apoptosis [40], and integrin-mediated cell adhesion [41] via exciting the cyclic adenosine monophosphate (cAMP) activating the downstream protein kinase pathway A (PKA) [42] enabling detached cells to survive and migrate. Importantly, β_2AR antagonists such as propranolol have been shown to inhibit norepinephrine-mediated angiogenesis and metastasis in vitro [37, 38, 43-45] and in vivo [46-48] and these benefits appear to extend to several cancer sites [49-51].

The role of β-blockers in cancer progression has been infrequently assessed. Early RCTs had suggested excess cancer mortality in relation to use of β-blockers [52-54], and this was confirmed in a meta-analysis of these three trials [55]; however, subsequent studies have refuted this showing β-blocker users to experience similar cancer mortality as non-users [56-58] a finding supported by a more recent meta-analysis of six RCTs [59] pooled OR 1.02 (95%CI 0.92, 1.14). In the most recent investigation amongst 466 patients with early stage (I & II) primary breast cancers, Powe *et al* [60] found a 71% reduction in breast cancer mortality HR 0.29 (95%CI 0.12, 0.72) and a 57% reduced risk of distant metastasis HR 0.43 (95%CI 0.20, 0.93) comparing β-blockers users vs. non-users; however, this analysis was based on a small number of β-blocker users (n=43). There are currently two clinical trials investigating the preventative role of perioperative propranolol and etodolac (COX-2 inhibitor) in cancer recurrence and progression in patients with breast (http://clinicaltrials.gov/ct2/show/NCT00502684) and colorectal cancer (http://clinicaltrials.gov/ct2/show/NCT00888797) undergoing surgery with curative intent.
Given the potential for adrenoreceptor antagonists to impede cancer progression [61] and mediate prognostic factors [50], a robust epidemiological investigation into the role β-blockers may play in cancer progression is warranted.

1.4 Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) and cancer progression

Recently, it has been suggested that the renin-angiotensin-aldosterone system (RAAS), which is critical in renal and cardiovascular homeostasis, may be implicated in the development of tumours. Angiotensin II is a polypeptide hormone which acts on angiotensin II type I receptors (ATIR) causing blood vessels to constrict, resulting in an increase in blood pressure and as such is thought to play a pivotal role in RAAS [35]. Angiotensin II is converted from angiotensin I by angiotensin converting enzyme (ACE), an enzyme which can be pharmaceutically inhibited via ACE inhibitors (ACEIs). ACEIs and angiotensin II type I receptor blockers (ARBs) are a group of widely prescribed pharmaceuticals

that are used primarily in the treatment of hypertension and congestive heart failure, left ventricular systolic dysfunction and to slow the progression to dialysis or transplantation in diabetic neuropathy.

ARBs function by blocking the activation of angiotensin II type I receptors. This is important because angiotensin II is a known growth factor and can stimulate tumour neovascularisation, an important requirement for tumour growth [62, 63] and suppression of this system might prevent cancer progression. In vitro and in vivo studies have demonstrated that ARBs and ACE inhibitors through selective inhibition of ATIRs decrease tumour growth, tumour-associated angiogenesis and metastasis [64, 65]. Despite biologically plausible mechanisms, epidemiological studies examining the role of ARBs and ACEIs in the prevention and progression of cancer are limited and results to date have been inconsistent.

ACE inhibitor use has been associated with a reduction in the risk of oesophageal, colon, lung pancreatic cancer and prostate cancer [66, 67] [68]. Other epidemiological studies have failed to find a protective effect for these agents. Interestingly, a recent meta-analysis of randomised controlled trials reported an increase in cancer incidence among regular users of ARBs [69]. A second meta-analysis refuted these findings but could not rule out a slight increase in cancer risk with combination use of ARBs and ACEIs [70].

Few studies have examined the association between use of these drugs and cancer progression. Wilop *et al* [71], retrospectively assessed long-term medication with ACEIs and ARBs amongst 287 patients with advanced non-small cell lung cancer undergoing chemotherapy. In multivariate analysis patients receiving either ACEIs or ARBs vs. non-recipients survived a median of 3.1 months longer HR 0.56. A further study by Chae *et al* [72], investigated the association between use of ACEIs or ARBs and the risk of tumour recurrence amongst asymptomatic BC patients (stage I-III). 23% of non-users developed a recurrence over a mean of 4.4 years follow-up vs. just 14% amongst users OR 0.54 (95%CI 0.33, 0.97). Five year disease free survival was also significantly higher in ACEIs/ARBs users vs. non-users; in addition use was associated with a reduction in mortality amongst a subset of patients with hypertension OR 0.41 (95%CI 0.23, 0.80) but not amongst all breast cancer patients. Overall, there is growing evidence that use of ACEIs and ARBs are associated with a reduced risk of cancer recurrence, however the number of patients treated with ACEIs/ARBs in these studies has been quite small. Larger observational studies are needed to compile evidence for the conduct of a more robust prospective randomised trial.

1.5 Northern Ireland enhanced prescribing database (NIEPD)

Under the provision of the National Health Service (NHS) in Northern Ireland (NI), all prescription medications are dispensed free of charge to the entire population, irrespective of age or means. Uniquely within the UK, in 2008 in NI the Business Services Organisation (BSO) implemented an Enhanced Prescribing Database (EPD) recording prescription and dispensing processes through the use of two-dimensional barcode technology. A prescription is generated electronically by the initial prescriber (the patient's GP) and printed onto the usual paper script. However each script is encoded with a 2D barcode (XML) containing the patient's name, address, postal code, date of birth, age, Health & Care number (H&C), GP's name, surgery name/address, name of the drug(s), instructions of use (one a day etc.), date of issue and the dose and quantity of the drug to be supplied. Ultimately it is intended that scripts will be scanned by community pharmacists when the patient or a nominated representative presents the script for collection of the medication, automatically collecting dispensing data. However at present the 2D barcodes are scanned at BSO when they are received from all pharmacies across NI at the end of each month. Thus whilst this offers assurance that prescriptions written by the GP have been dispensed, there is as yet no way of assessing individual compliance and usage of this medication. At present approximately 90% of all prescriptions scanned at BSO results in useable data in EPD. A central database of prescribed and dispensed drugs for approximately 1.9 million patients registered with a GP in NI now exists in BSO for use by healthcare professionals and researchers.

1.6 Northern Ireland Cancer Registry

The Northern Ireland Cancer Registry (NICR) was established in 1994 and uses an automated computer system with multiple information sources to collate information on new diagnoses of cancer, with information collected for incidence from 1993 onwards. The three main sources for registration are the Patient Administration System (PAS) used by all hospitals, histopathology reports and death notifications which are supplied by the General Registrar Office (GRO). From PAS, the registry obtains demographic information on individual patients along with basic site and behaviour information (benign or malignant) for each tumour. This information is supplemented by electronic downloads from histopathology and cytopathology laboratories i.e.: specification of histological tumour grade (specifies degree of cell differentiation and is an indicia of tumour aggressiveness) and morphology (microscopic histopathological diagnosis by a pathologist). A major focus of the registry's work is on the verification of information from a single hospital admission, a single histopathology report or a single death certificate (death initiated cases). Trained Tumour Verification Officers (TVOs) examine general practitioners' (GPs) notes for patients who have died

from cancer, hospital records for cases identified without histopathology or cytology confirmation and histopathology reports where there is conflicting information or other possible errors. Follow-up of patients is conducted passively by linking cancer incidence data to death certificate information. Data on cancer mortality also comes from the information supplied by GRO.

2.0 Plan of investigation:

The proposed investigation will involve establishment of three retrospective cohorts of confirmed BC, CRC and LC cases diagnosed between 2008 and 2011 from the Northern Ireland Cancer Registry (NICR) and subsequent linkage of these cohorts to pertinent prescription and dispensing data held within the Northern Ireland Enhanced Prescribing Database (NIEPD).

2.1 Aims and objectives

To investigate if regular use of non-steroidal anti-inflammatory drugs (NSAIDs), angiotensin converting enzyme Inhibitors (ACE inhibitors), angiotensin receptor blockers (ARBs), beta-blockers (β-blockers) or bisphosphonates, defined as ≥ 3 times per week for one month or more, is associated with disease progression in breast (BC), colorectal (CRC) and lung cancer (LC) patients.

Specific hypotheses will be to test whether or not:

- regular post-diagnostic NSAID use is associated with a reduction in BC, CRC and LC specific or all-cause mortality and cancer recurrence
- regular post-diagnostic ACE inhibitors/ARB use is associated with a reduction in BC, CRC and LC specific or all-cause mortality and cancer recurrence
- regular post-diagnostic β-Blocker use is associated with a reduction in BC, CRC and LC specific or all-cause mortality and cancer recurrence
- regular post-diagnostic bisphosphonate use is associated with a reduction in BC, CRC and LC specific or all-cause mortality and cancer recurrence

2.1 Methodology:

2.1.1 Study design

This study will utilise a nested case-control approach to cohort analysis to investigate cancer survival and progression in BC, CRC and LC patients. The process of NICR-EPD data linkage will occur at two main time points. The first linkage will be performed in early 2012, prescribing and dispensing data from BSO will be taken from the 1st July 2008 (i.e.: date from which the best quality prescription and dispensing data has been collected) to the latest available date prior to linkage. This data will then

be merged with cancer staging and treatment data captured for the same period. This means that for the first linkage follow-up will run from the 1st July 2008 until at least the 31st Dec 2011 (approximately 3.5 years). For a number of subjects, particularly amongst those individuals with early stage disease at diagnosis, this follow-up may be insufficient for study outcomes (cancer deaths/recurrences) to occur; therefore this first linkage will be viewed as an exploratory investigation and will provide a chance to develop and refine the data handling, cleaning and analysis that will we used in the final dataset. Data linkage will be repeated on a second occasion; this will be an entirely new linkage extending the follow-up of study outcomes up to the end of 2013 i.e.: an additional 24 months of follow-up, providing a maximum cohort follow-up time of 5.5 years (1st July 2008 – 31st Dec 2013).

2.1.2 Study population

We plan to establish three cohorts of all incident (newly occurring) primary BC, CRC and LC patients using incidence data obtained through the NICR. All cancer cases diagnosed from 1st July 2008 until at least the 31st Dec 2011 will be included in the cancer cohorts (i.e.: a minimum of 3.5 years of cancer incidence data will be collected). Individuals with any stage of BC, CRC and LC will be considered eligible for the study. Male BC patients will be excluded, but both male and female CRC and LC cases will be eligible. Patients aged ≤18 years and those in which a prior cancer diagnosis (other than non-melanoma skin cancer (NMSC)) has been made will also be ineligible.

1156, 1108 and 1007 incident cases of BC (female only), CRC (all persons) and LC (all persons) were diagnosed in Northern Ireland in 2008 [73]. In the NICR for the same year, staging for BC, CRC and LC was 89.5% and 81.7% complete respectively; the percentage of LC patients with available staging information was much lower at 45.6% on average. In terms of stage of disease at diagnosis, in 2006 in Northern Ireland there were 951 incident BC cases, of which 27.9%, 32.4%, 17.8% and 7% were stage I-IV disease respectively [74]. In the same year there were 913 incident CRC cases, 10%, 27%, 26% and 25% of which were stage I-IV (Duke's A-D) disease [75]. 834 incident LC cases were reported in 2006 and 13%, 5%, 16% and 48% were stage I-IV at diagnosis [76].

Therefore given a minimum of 3.5 years of incidence data being collected for each cancer cohort and accounting for the percentage of likely staged cancers in each year, it is estimated that 8398 patients with any stage disease (3623 BC, 3168 CRC and 1607 LC cases) will be available for analysis. Of these 8398 patients, it may be expected that 2185 BC, 1172 CRC and 289 LC cases (3646 cases in total) will be early stage disease (TNM I-II) at the time of their initial diagnosis.

2.1.3 Data sources

All primary incident breast, colorectal and lung cancers diagnosed from the 1st July 2008 until the point of first data linkage will be identified through the NICR. Data extraction will be undertaken by tumour verification officers (TVOs) employed within the NICR. These individuals will utilise pre-defined data extraction forms to populate each cancer specific database with tumour staging and treatment information and other relevant information.

Staff at BSO will be responsible for creating a database of commonly prescribed drugs (Annex 1) – this will be constructed independently of the research team. It is envisaged that the database from the NICR will be linked to the dataset in the BSO via each patient's Health & Care Number using a one way encryption technique; methods for this process have been detailed in Annex 3 and the data linkage is outlined in 2.1.3.1 below. A list of available but irrelevant prescriptions (i.e.: scripts for bandages etc.) from the EPD data is shown in Annex 2. These items will not form part of the NIEPD data request from BSO. As aforementioned, roughly 90% of all prescriptions scanned at BSO result in usable data, however in early 2008 there were several months involving adaptation (printer and software installation throughout GP practices) to the new electronic system wherein a less optimal scan rate may have been obtained. Therefore as a measure of data quality, only those GP practices with a script scan rate of ≥70% will be included in the final study. As BSO receives weekly updates of deaths from the General Registrars Office (GRO), information pertaining to the fact of death (all-cause mortality) will be obtained under the auspices of the BSO. Subject to a data access agreement information on the initial cause of death and or the ICD coded cause of death (cancer specific mortality – obtained several months in arrears) may also be obtained. BSO staff will also use each patient's super output area to generate a deprivation quintile (described in 2.1.6 below).

2.1.3.1 Process of data linkage

First linkage (01/07/2008 – to the latest available date prior to linkage) – exploratory study

Step 1: NICR staff will provide staff at BSO with a list of one-way encrypted H&C numbers from incident BC, CRC and LC cases newly diagnosed over this period. Among these patients approx 1000 will be individuals who have been diagnosed with premalignant conditions, thus adding 'noise' to those with a real diagnosis of cancer. This process ensures that staff at BSO will not receive any treatment or staging information at any point and will not be able to distinguish between those individuals with a premalignant condition and those with actual cancer.

Step 2: Staff at BSO will then use the same one-way encryption algorithm to encrypt all the H&C numbers held at BSO. Matching H&C numbers from both the NICR and BSO will be linked to all

prescribing and dispensing data held for these persons from the 01/07/2008 to the latest available date prior to the linkage.

Step 3: The EPD data will be transported to the NICR (via encrypted e-mail or CD). The encrypted H&C number will be attached to the EPD dataset. This will be linked to the encrypted H&C number held in the NICR. The two datasets will then be merged and the encrypted identifiers deleted from each dataset (described in detail in Annex 3). To ensure that the encryption process cannot be reversed on record order, each dataset will be sorted in a random order.

Second linkage (01/01/2012 – 31/12/2013) – Subsequent follow-up

Step 1: To follow-up cancer deaths and recurrences, NICR staff will perform a fresh one-way encryption on the H&C numbers and cancer treatment and staging data will be updated. The process is then repeated as in steps 2 and 3 above. This process of encryption maximises patient anonymity ensuring that the research team and others are unable to identify individual patients from either dataset.

2.1.4 Outcomes

The principal outcomes are cancer-specific and all-cause mortality in accordance to drug use. Data on the date of occurrence and cause of death will be available via the GRO, information that will be obtained under the auspices of the BSO. Comparison of associations between intended drug exposures and cancer-specific and all-cause mortality will facilitate an assessment of whether any apparent protective effect against cancer progression results from a healthy user effect.

Data on cancer recurrences i.e.: local (in the vicinity of the primary), regional (in surrounding lymph nodes) and metastatic recurrence (spread to another organ/tissue) will be obtained from the NICR prior to linkage of the NIEPD data and will be defined as a relapse of the primary cancer after a period in which no cancer could be detected (this time-frame will vary from site to site and from person to person so cannot be clearly defined). Cancer recurrence will be a secondary outcome measure for this study.

2.1.5 Exposures

The principal exposures of interest will be the use of NSAIDs and aspirin (including low dose aspirin (75mg)), angiotensin converting enzyme Inhibitors (ACE inhibitors), angiotensin receptor blockers (ARBs), beta Blockers (β-blockers) and bisphosphonates post-diagnosis of BC, CRC and LC; a generic list of relevant drugs has been detailed in Annex 1. In sensitivity analysis we will exclude NSAID/aspirin use in the first 6 months after cancer diagnosis to assess differences in drugs indicated

for pain related to initial cancer symptoms and treatment. Similarly, the sensitivity of excluding common drug use in the 6 months preceding death or disease recurrence (and a corresponding period in matched controls) will be examined to account for drugs used in symptom palliation (sensitivity analyses will be conducted to investigate the resulting effect size when varying this time interval).

Data derived from the scanned prescription (i.e.: the quantity, dose and frequency of drug use) and the date of prescription will be used to assign a Defined Daily Dose (DDD). DDDs are a validated statistical method of drug consumption maintained by the World Health Organization [77] and can be defined as the assumed average dose per day of a drug used for its main indication in adults.

2.1.6 Covariates

Potential confounders for this analysis may relate to lifestyle factors thought to be associated with BC, CRC and LC survival including, smoking status, alcohol consumption, Body Mass Index (BMI), diet and physical activity level [78-81]. Some of these data may be available from the NICR. However data on diet and physical activity is lacking and anthropometric data such as height and weight are not always available. It's important to remember however that although these factors may be associated with cancer survival, it is less clear that they are associated with the drug exposures that are of interest in this study and therefore may not be true confounders.

Comorbidity may be associated with the use of various drugs, particularly those with a cardiovascular indication, and are therefore also likely to have an impact on mortality and access to treatment. Information on comorbidities will be available from NICR for some (but not all) cases. Socioeconomic status also has the potential to impact on disease survival possibly modifying health behaviours, access to services and drug exposure. At BSO, each patient's super output area will be ascertained using the Central Postcode Directory (CPD) which is annually updated by the Northern Ireland Statistics and Research Agency (NISRA). This will be used as to assign each patient record with a deprivation score based upon the economic characteristics of all persons usually resident in that area [82].

Aromatase inhibitors have the potential to induce joint pain which in turn may increase the use of NSAIDs to palliate this symptom. It is also thought that aromatase inhibitors may improve BC survival [83]; as such they are best regarded as both an effect modifier and a confounder. There are several other drugs which will need to be considered as covariates in specific drug analyses, for example bisphosphonate users may have an increased use of NSAIDs to alleviate joint pain and swelling,

moreover bisphosphonates may prevent BC progression [30] and so should be considered as confounders in the analysis of NSAIDs.

2.1.7 Statistical Analysis

2.1.7.1 Nested case-control study

The BC, CRC and LC cohorts will be analysed as nested case control studies, the outcome of interest will be death from BC, CRC or LC, all-cause mortality and disease recurrences. The nested case-control design offers a highly efficient epidemiological approach to the assessment of exposure-disease associations and is an established method for computational reduction in comparison to Cox regression [84]. Compared with time-varying survival analysis of cohort data, this method will produce unbiased effect estimates with minimal loss of precision [85] by better controlling for potential confounding variables and improved quantification of exposure with respect to time [86]; moreover the nested case-control design overcomes the issue of immortal time bias [86, 87]. Immortal time bias refers to a period of follow-up in cohort analysis in which study outcomes cannot occur. For example, in a traditional cohort analysis of this study there may be a delay in prescription of a drug (exposure of interest) after the initial cancer diagnosis has occurred (start of study follow-up). This risks biasing effect estimates in favour of the exposure under study, as a spurious survival advantage may be seen amongst those receiving the drug under observation [87, 88].

Similar analytical strategies will be used in all three cohorts, for example, in the BC specific survival analysis a time-matched nested case-control analysis will be performed. Cases will be defined as cohort members who have died from BC or who have disease recurrence and will be matched to up to 5 patients alive and free of disease recurrence/progression at their time of death (defined as the controls). Controls will be matched on age (in 5-year intervals) and year of breast cancer diagnosis; a form of incidence density sampling which involves matching each case to a sample of those patients who are at risk at the time of case occurrence. Hence the index date for each case will be defined as the date of death/recurrence and this will be allocated to each matched control. The conceptually relevant drug exposure period for the main survival analysis will be 12 months following BC diagnosis to the 12 months prior to the index date in both the cases and controls.

Conditional logistic regression analyses will be conducted initially to calculate the odds of death and 95%CI for those ever exposed and those never exposed to each of the drugs of interest. Separate analysis will be conducted to examine regular drug use (≥3 times per week for 1 month or more) and duration of use (in DDDs). These analyses will be adjusted for the potential confounders detailed in 2.1.6 above; confounders with missing data will be incorporated using a missing data category.

Analyses will be stratified on age, menopausal status (in BC cohort analysis), gender and site (CRC and LC cohorts). Provided the follow-up period is long enough for cancer specific deaths and recurrences to occur, analyses stratified by cancer stage may also be undertaken.

In order to discern whether associations with NSAID/aspirin use are merely a reflection of a non-specific analgesic effect we plan to assess regular paracetamol use (unassociated with disease progression but with an analgesic effect) and compare these recurrence and survival benefit results. All statistical analyses will be performed using STATA version 11.0 (StataCorp LP, College Station, TX, USA), all tests will be 2-sided with the level of significance set at the 5% level.

2.2 Sample size:

In a recent prospective study in the US, the proportion of breast cancer patients taking aspirin and other NSAIDs regularly (3 or more days/wk) was reported as 27% [6]. Given a minimum of 3.5 years of cancer incidence data being collected from the NICR and examining Northern Ireland breast cancer survival data [73], over 450 breast cancer specific deaths can be expected to occur. Based upon matching 5 controls to each cancer death (as described in 2.1.7.1 above) and approximating 27% use of NSAIDs amongst breast cancer patients who have not died over the study period, we will have over 80% power at the 95% confidence level (alpha 0.05, two sided) to detect around a 30% reduction in risk of BC mortality in NSAID users vs. non-users.

2.3 Limitations of the study:

A particular difficulty with any pharmacoepidemiology study is the issue of confounding by indication. In observational studies of drug effects there is no randomisation of individuals who are users or non-users of the drug under observation; this is particularly true of widely used over-the-counter and prescription drugs (i.e.: analgesics) as the indication for treatment may be related to prognostic factors or future health outcomes generating an underlying risk profile imbalance. For example, lower Bone Mineral Density (BMD) is an indication for bisphosphonate use but is also associated with lower BC incidence, as lifetime exposure to oestrogen influences not only breast cancer risk but also BMD [89]; therefore BMD and breast cancer risk may be related. Consequently women in whom bisphosphonate therapy would be initiated might represent a lower risk group for breast cancer than women with normal BMD. This problem may be minimised by controlling for known prognostic factors. Sensitivity analyses of exclusion of observed drug use in the period immediately after cancer diagnosis and in the period preceding cancer death/recurrence will also help reduce this bias. Although 90% of all prescriptions scanned at BSO result in usable data and only GP practices with a script scan rate of over 70% will be included in the final dataset, all analyses will

be conducted on the assumption that the medications dispensed from the community pharmacies have been taken as directed; it will not be possible to assess individual compliance with the prescribed medication.

2.4 Project timetable:

Provided that ethical consent and governance approval for this study is granted before Autumn 2011, it is anticipated that the initial stage of this study (extraction of data from NICR) will require 6 months to complete i.e.: a total of 2 months for each of the three cancer sites investigated bringing the study up to February/March 2012. At this stage follow-up from the cancer cohorts should be from the 1st July 2008 – 31st Dec 2011 (i.e.: a maximum of 3.5 years of total follow-up). A further month may be required to match the encrypted health and care numbers from each of the three NICR datasets to prescription and dispensing data held on the EPD database. The first linkage of the NICR and EPD data should therefore occur around April 2012. At this point an initial exploratory analysis will be conducted. A period of several months will be allotted to allow further follow-up data (cancer deaths and recurrences and further treatment information) to be compiled by the NICR. Follow-up for all cancer patients is expected to be completed by the end of 2013, providing a maximum follow-up period of 5.5 years for those cases diagnosed in early July 2008. At this stage a fresh linkage will be made between the follow-up data from the NICR and prescription/dispensing data in BSO. Analysis and interpretation of the full dataset will be completed by autumn 2014. It is therefore envisaged that this project will be approximately 3 years in duration.

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Annex 1
Generic names of commonly prescribed drugs in the UK which will be used in the NIEPD_NICR study

NSAIDs:

Indometacin, sulindac, diclofenac, etodolac, acemetacin, accelofenac, piroxicam, tenoxicam, meloxicam, ibuprofen, naproxen, ketoprofen, fenoprofen, fenbufen, flurbiprofen, tiaprofenic acid, dexibuprofen, dexketprofen, mefanamic acid, tolfenamic acid, celecoxib, etoricoxib, lumiracoxib, nabumetone, azapropazone, aspirin

B-Blockers:

Betaxolol, levobunolol, metipranolol, carteolol, Acebutolol, atenolol, atenolol in combination with calcium-channel blocker (i.e.: Beta-Adalat, Tenif), bisoprolol, carvedilol, celipropolol, esmolol, labetalol, metoprolol, nadolol, nebivolol, oxyprenalol, pindolol, propranolol, sotalol, timolol.

ACE inhibitors:

Captopril, cilazapril, enalapril maleate, fosinopril sodium, imidapril hydrocholoride , perindopril erbumine, quinapril, ramipril, trandolapril, lisinopril, moexipril

Angiotensin-II receptor antagonists:

Candesartan cilexietil, eprosartan, irbesartan, losartan potassium, olmesartan medoxomil, telmisartan, valsartan

Bisphosphonates:

(Oral indications only)

Alendronate, sodium clodronate, disodium etidronate, ibandronate, risedronate sodium, disodium tiludronate

Annex 2

List of BNF codes/ categories to be excluded from EPD data

7.4.4 Bladder Instillations and urological surgery
- 1. Sterile sodium citrate solution for bladder irrigation
- 2.Glycine irrigation solution
- 3. Catheter patency solutions
  - -chlorhexidine 0.02%
  - -sodium chloride 0.9%
  - - Solution G (Uriflex G, Uro-Trainer Twin Suby G)
  - -Solution R (Uriflex R, Uro-trainer Twin Solution R)

9.5.3 Fluoride
- -Tablets
  - En-De-Kay (also oral drops)
  - Fluor-a-day
  - Fluorigard
- -Mouthwashes
  - Duraphat
  - En-De-kay
  - Fluorigard
- -Toothpastes
  - Duraphat

9.6.7 Multi-vitamin preparations
- -vitamin capsules
- -abidec/dalivit drops
- -Forceval
- -Ketovite

12.1.3 Removal of Ear wax
- -almond oil
- -olive oil
- -sodium bicarbonate
- -cerumol
- -exterol
- -Molcer
- -Otex
- -Waxsol

13.2.2 Barrier preparations
13.8.1 Sunscreen preparations
13.8.2 Camouflagers
13.11.1 Alcohols and saline
13.11.2 Chlorhexidine salts
13.11.3 Cationic surfactants and soaps
13.11.4 Iodine
13.11.5 Phenolics
13.11.6 Oxidisers and dyes
14.4 vaccines and antisera

Appendix 7:Nutritional products and gluten free products
Appendix 8:Wound management products and elasticated garments
Ostomy/ urinary equipment as described in the NI Drug Tariff.

Annex 3

One-way encryption process:

An integral component of the NICR-NIEPD data linkage is the use of each individuals Health and Care number as the unique identification field. An important concern and potential ethical issue of the NICR-EPD data linkage is that of data confidentiality and the potential identification of individuals or patients from this field in the final dataset.

We plan to use an Advanced Encryption Standard 256 bit (AES 256) algorithm to conduct a one way encryption of each patient's H&C number, undertaken sequentially on each dataset. The encryption process has been summarised in the flowchart below. Briefly, H&C numbers from the NICR and BSO will be fed into the data encryption software. The data will be arranged such that the first 10 characters will correspond to each individual's unique H&C number. The encryption process requires two distinct text strings, the first is a password or cipher which forms the basis of the encryption, the second string is additional text which pads the characters out for a more secure encryption. Both text strings will only be known by data custodians in BSO and the NICR where the encryption will be undertaken; without these two strings it is impossible for the researcher to invert the coded data and the process cannot be replicated at a later date. The result is a new encrypted unique identifier ensuring that the remaining variables are dissociated from identifiable data. To ensure that the encryption process cannot be reversed on record order each dataset will be sorted in a random order. The two datasets will then be merged using the unique encrypted identifier present on both datasets, before finally removing this field from the final data file. The final file will then be made available to the approved researcher(s) in a secure setting for analysis i.e.: held in a secure office on a password protected PC with access restricted to the intended researcher(s).