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# ANTI-TNF THERAPY AND MALIGNANCY IN PATIENTS WITH RHEUMATOID ARTHRITIS: STUDIES ON INCIDENCE, RECURRENCE AND SURVIVAL

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Anti-TNF therapy and malignancy in patients with rheumatoid arthritis: studies on incidence, recurrence and survival

# THESIS FOR DOCTORAL DEGREE (Ph.D.)

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# ABSTRACT

Tumor necrosis factor inhibitors (TNFi) have become a backbone treatment of rheumatoid arthritis (RA). TNF has multiple and incompletely understood functions in tumor biology, and cancer is considered a potential adverse event of TNFi treatment. The overarching aim of this thesis was to investigate the risk-benefit balance in RA-patients treated with TNFi, focusing on skin cancer, breast cancer progress and post-cancer survival. To put the risks into context we also contrasted RA-patients never treated with biological drugs (biologics-naïve) to the general population. We used data from medical files, national health and census registers and the RA quality of care register, to define clinically relevant subsets of RA and cancer-related outcomes among them.

**In study I** we investigated the risk of malignant melanoma and all-site cancer in TNFi-treated RA-patients (1998-2010), biologics-naïve RA-patients, and matched general population comparators. We detected a 50% increased risk of invasive malignant melanoma, but no increased risk of *in situ* melanoma or all-site cancer among TNFi-treated compared to biologics-naïve RA-patients.

**In study II** we investigated the risk of non squamous cell cancer (SCC, 1998-2011) and basal cell cancer (BCC, 2004-2011) in TNFi-treated RA-patients, biologics-naïve RA-patients, and matched general population comparators. We found a 20% increase in risk of *in situ* SCC among TNFi-treated compared to biologics-naïve RA-patients, but no increased risk of BCC. In biologics-naïve RA-patients, we detected a doubled risk of SCC, and a 20% increased risk of BCC compared to the general population**.**

**In study III** we investigated the risk of breast cancer recurrence in 120 female RA-patients who started TNFi treatment (1999-2010) on average a decade after diagnosis of breast cancer. As comparator we used 120 biologics-naïve RA-patients with a history of breast cancer, matched on sex, age, year and cancer stage at diagnosis, and residency. We found no difference in risk of recurrent breast cancer and all-cause mortality between the two groups, after adjusting for breast cancer related prognostic factors.

**In study IV** we investigated the clinical stage at diagnosis, and post-cancer survival of cancers developing during or after TNFi treatment (1999-2007), compared to cancers among biologics-naïve RA-patients. We used both a matched and an unmatched approach. No major differences in cancer stage at diagnosis or in post-cancer survival were observed among TNFi-treated RA-patients, compared to biologics-naïve RA-patients with cancer.

# LIST OF SCIENTIFIC PAPERS

I. **Pauline Raaschou**, Julia F Simard, Marie Holmqvist, Johan Askling, For the ARTIS study group.

Rheumatoid arthritis, anti-tumour necrosis factor therapy, and risk of malignant melanoma: nationwide population-based prospective cohort study from Sweden.
BMJ 2013 Apr; 346, fl939

II. **Pauline Raaschou**, Julia F Simard, Charlotte Asker-Hagelberg, Johan Askling for the ARTIS study group.

Rheumatoid arthritis, anti-tumour necrosis factor therapy, and risk of squamous cell and basal cell skin cancer- a nationwide population-based prospective cohort study from Sweden
In manuscript

III. **Pauline Raaschou**, Thomas Frisell, Johan Askling for the ARTIS study group

TNF inhibitor therapy and risk of breast cancer recurrence in patients with rheumatoid arthritis -a nationwide cohort study.
ARD Online First, published on August 8, 2014 as 10.1136/annrheumdis-2014-205745

IV. **Pauline Raaschou,** Julia F Simard, Martin Neovius, Johan Askling, For the ARTIS study group

Does cancer that occurs during or after anti-TNF therapy have a worse prognosis? A national assessment of overall and site-specific cancer survival in rheumatoid arthritis patients treated with biologics.
Arthritis & Rheumatism 2011 Jul; 63(7): 1812-22

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACPA | Anti-Citrullinated Protein Antibody |
| ACR | American College of Rheumatology |
| ARTIS | Anti-Rheumatic Treatment in Sweden |
| AS | Ankylosing Spondylitis |
| BCC | Basal Cell Cancer |
| bDMARD | Biologic Disease Modifying Anti Rheumatic Drug |
| CDAI | Clinical Disease Activity Score |
| CIE | Commission Internationale d´Eclairage |
| CNS | Central Nervous System |
| COPD | Chronic Obstructive Pulmonary Disease |
| CRP | C-Reactive Protein |
| csDMARD | Conventional Synthetic Disease Modifying Anti Rheumatic Drug |
| DAS28 | Disease Activity Score 28 joints |
| DDD | Defined Daily Dose |
| EMA | European Medicines Agency |
| EULAR | The European League Against Rheumatism |
| HAQ | Health Assessment Questionnaire |
| HLA | Human Leukocyte Antigen |
| HIV | Human Immunodeficiency Virus |
| ICD | International Classification of Diseases |
| IL | Interleukine |
| JAK | Janus Kinase |
| JIA | Juvenil Idiopathic Arthritis |
| MAPK | Mitogen Activated Protein Kinase |

| | |
|---|---|
| MHC | Major Histocompatibility Complex |
| MEK | Mitogen activated protein Kinase |
| MSD | Musculoskeletal Disorder |
| NBHW | National Board of Health and Welfare |
| NK-cells | Natural Killer cells |
| NMSC | Non-melanoma Skin Cancer |
| NPR | National Patient Register |
| OTC | Over The Counter |
| PASS | Post Authorization Safety Study |
| PDR | Prescribed Drug Register |
| PIN | Personal Identification Number |
| PRAC | Pharmacovigilance and Risk Assessment Committee |
| PsA | Psoriatic Arthritis |
| RA | Rheumatoid Arthritis |
| RF | Rheumatoid Factor |
| RMP | Risk Management Plan |
| SCC | Squamous Cell Cancer |
| SDAI | Simple Disease Activity Index |
| SLE | Systemic Lupus Erythematosus |
| SR | Sedimentation Rate |
| SRQ | Swedish Rheumatology Quality of care register |
| TNFi | Tumor Necrosis Factor inhibitor |
| TNFR | Tumor Necrosis Factor Receptor |
| TNM | Tumor Node Metastasis |
| UV-radiation | Ultraviolet radiation |

# 1 INTRODUCTION

TNF is a key mediator of the inflammatory response. Blocking of TNF by TNFi treatment is a mainstay in the treatment of several chronic inflammatory diseases including RA. TNF is also relevant in carcinogenesis and tumor progression. It is presumed to affect several steps in the origin and development of cancer via mechanisms that are incompletely understood and presumed to have varying significance depending on the cancer type. Accordingly, there is a concern that TNFi treatment might affect both the clinical occurrence as well as prognosis of cancer.

The introduction of powerful pharmaceuticals such as TNFi and other biological drugs poses new challenges in pharmacoepidemiology. Traditional small peptide drugs typically target a specific receptor, enzyme, or ion-channel, which translates into predictable target effects and adverse events. Biological drugs exhibit broad and largely unknown pharmacodynamic effects without a clear-cut definition of effects and side-effects. Any side-effects of such drugs will be intimately related to the specific disease, the severity of the disease, the presence of comorbidities and use of concomitant medications [1-2]. In view of this complexity, the safety-profile of TNFi is bound to be variable and related to the patients under study. For generalizability of the findings it is important to identify a clinically relevant study population. Ideally, it should reflect the patients actually seen by the physician in the context where research meets clinical reality.

The cohort study design is well suited for the investigation of serious, uncommon events among individuals selected for a treatment in clinical practice. Many of the features of the Swedish health information network provide an ideal setting for such studies. In this thesis I have used data from medical files, national health and census registers and the RA quality of care register, to define cancer related outcomes among clinically relevant subsets of RA who were treated and not treated (biologics-naïve) with biologic drugs.

# 2 AIMS

The overarching aim of this thesis is to investigate the risk-benefit balance in RA-patients treated with TNFi, focusing on malignancies, in particular: skin cancer incidence, breast cancer recurrence and post-cancer survival. To put the risks with TNFi into context we also compared biologics-naïve RA-patients with the general population.

**Study I** Our aim was to investigate the risk of malignant melanoma of the skin and all-site cancer in patients with RA compared with the general population and whether TNFi treatment had any further impact on the risk.

**Study II** Our aim was to investigate the risk of first SCC and BCC in biologics-naïve patients with RA compared to the general population, and whether TNFi treatment had any further impact on the risk.

**Study III** Our aim was to investigate the risk of breast cancer recurrence in female TNFi-treated RA-patients, compared to the corresponding risks in matched biologics-naïve patients with RA, while adjusting for clinical features of the breast cancer.

**Study IV** Our aim was to investigate the influence of TNFi on the cancer stage at diagnosis and to determine survival rates following cancer, among TNFi-treated compared with biologics-naïve patients with RA.

# 3 BACKGROUND

## 3.1 REGISTER-BASED STUDIES IN SWEDEN

Sweden has a population of 9.6 million individuals [3]. Sweden's long tradition of keeping regional and national registers of births, deaths, migrations and health, makes it an ideal setting for register based studies. A personal identification number (PIN) is issued to all Swedish citizens and legal residents with permission to live in Sweden for at least one year [4]. Using the PIN, researchers can gather information about residency, vital status, treatment and relevant outcomes through linkages of national and virtually complete administrative and clinical registers. The National Board of Health and Welfare (NBHW, Socialstyrelsen) and Statistics Sweden (Statistiska Centralbyrån) are the two major authorities governing the national health and census registers [3, 5].

## 3.2 REGISTERS USED IN THIS THESIS

### 3.2.1 Registers of rheumatoid arthritis

#### *3.2.1.1 The Swedish Rheumatology Quality of care register (SRQ)*

SRQ is one of seven quality of care registers in Sweden with the highest rank, based on quality and management [6]. It was initiated under the auspices of the Swedish Rheumatology Association in 1996 in order to collect clinical data on a patient-level basis and to provide a basis for quality management and research (during 2013, SRQ-data generated almost 40 research publications) [7]. Both health-care professionals and patients (since 2004) enter information such as functional status, disease activity and adverse events, via a web-based tool [7]. Within the SRQ, an inception cohort of early arthritis is nested, including individuals with RA symptoms of less than 1 year duration [7-9]. Currently 100% of Swedish rheumatology treatment facilities are linked to the register [7] .

#### *3.2.1.2 The Anti Rheumatic Treatment in Sweden Register (ARTIS)*

Approximately one third of individuals diagnosed with RA in Sweden today receive biologic treatment at some time-point [10]. The high potency of these drugs may lead to substantial treatment effects, but also potentially severe adverse events and high costs*. A clinical context with monitoring of effects, side-effects, and patient/societal economic burden is important to understand the overall value of a drug. ARTIS is a research database of biologics treatment nested within SRQ [9, 11-12]. It contains data on treatment efficacy, adverse events and drug retention of biological drugs used in the clinical care of adult patients with RA and other chronic inflammatory diseases. The aim of ARTIS is to provide information for treatment optimization in the individual patient, and for quality improvement and research.

** The total drug cost in Swedish rheumatology amounts to 211 million Euros annually [13], which equals 6% of the total Swedish drug cost.*

The national coverage of biologic treatment in RA in SRQ is close to 90% [12]. At treatment start and at follow-up visits, details on the underlying diagnosis, specific drug and dose, concomitant csDMARDs and oral steroids, as well as 28 joint Disease Activity Score and Health Assessment Questionnaire scores, are entered by the treating rheumatologist and the patient.

### 3.2.2 National health and census registers

#### *3.2.2.1 The National Population Register*

The National Population Register is updated daily and includes information on birth, death and burial site, residency (country, county, parish), migration, emigration/immigration and civil status of all residents in Sweden since 1961 [3]. Information in the register is distributed from a central database at the Swedish Tax Agency to central and regional authorities who use the data [14].

#### *3.2.2.2 The Cause of Death Register*

The Cause of Death Register is managed by the NBHW and provides information on dates and primary and contributing causes of death for all deceased residents 1961 and onwards [15]

#### *3.2.2.3 The National Health Registers*

The six national health registers in Sweden are managed by the NBHW [16] and three of these are used in this thesis (the National Patient Register, the National Cancer Register, and the National Prescribed Drug Register). The national health registers provide structured and high quality data with almost complete coverage for research on the usage and quality of the health care system and the epidemiology of diseases [17]. The registers can be used for research and statistics/quality control according to *(SFS 1998:543)* and Personuppgiftslagen *(SFS 1998:204)*.

The National Patient Register

The National Patient Register was initiated in the 1960´s by the NBHW and coverage was gradually increasing to cover patients from more specialties and more county councils [18-19]. From 1987 coverage was nationwide for inpatient care. The Swedish Outpatient Register was initiated in 2001 as a new component of the The National Patient Register. The Outpatient Register includes information on diagnoses in non-primary outpatient care, coded according to ICD version 10 [20]. The coverage varies with calendar year and specialty but is estimated to nearly 90% of all RA in Sweden. Missingness in the outpatient register stems primarily from non-somatic care [21].

The National Cancer Register

The National Cancer Register (established in 1958) is mandatory for the physician detecting the cancer as well as for the pathology departments verifying the diagnosis. Coverage is high, with an estimated overall under-reporting of 5% [22-23]. The registry contains data on cancer diagnosis date, cancer type (using the ICD classification [20]), and morphologic features. For most solid cancer sites, the tumor stage at diagnosis using the TNM system [24-25] has been reported to the registry since 2003.

The National Prescribed Drug Register

The National Prescribed Drug Register contains information on all prescribed (but not OTC) drugs dispensed to individuals at any Swedish pharmacy from July 2005 and onwards, with an estimated coverage of close to 100%. In addition to PIN, variables include dispensed item and dispensed amount measured in prescriptions and DDD [26-27].

#### *3.2.2.4 The Register of Education*

The Register of Education is managed by Statistics Sweden (Statistiska CentralByrån). It is updated annually to contain the highest level of education for each individual (primary secondary school education, adult education, undergraduate education or postgraduate education) from 1985 and onwards. Information on education is missing for around 1.5% among individuals aged 25-64 [28].

#### *3.2.2.5 The multi-generation register*

The multi-generation register is a part of the National Population Register and contains all individuals born in 1932 or later (index persons) and registered in Sweden at any time since 1961, with information on their biological and adoptive parents (and thereby also siblings) [3, 29]. The register coverage of index persons is virtually complete and the proportion of index persons with links to both parents is above 80%.

## 3.3 ETHICS IN REGISTER-BASED STUDIES

Any research involving humans has potential ethical dilemmas. In clinical experiments, these dilemmas might be more obvious and significant, than in register-based research. In both situations good research conduct is vital to safeguard the well-being of the study participants. In register-based research the principal ethical dilemma is the balance between maintaining the personal integrity of the study subjects, while allowing the researchers to handle data on personal matters such as health status. Personal integrity and autonomy are central concepts.

*Integrity*
The word integrity stems from the Latin word "integritas", meaning undamaged. It is used in philosophy, psychology, medicine, law, art and in other fields or sciences for the concept of "complete". There is no clear-cut definition of personal integrity in the context of medical research despite the term being used so frequently, but integrity is often used interchangeably with "human dignity" [30]. In psychology, to respect a person's integrity is "not to breach the wall of defense that people normally raise, to protect the most private sphere from interference or intrusion" (*my translation*) [31].

The conduct of medical research in Sweden is dictated by a set of rules including ethical codices [32-33], national and international guidelines without legal jurisdiction [34], as well as formal laws (including, but not limited to: Lagen om Hälsodataregister *(SFS 1998:543),* Personuppgiftslagen *(SFS 1998:204)* and Etikprövningslagen *(SFS 2003:460)).*

Every researcher in the medical field is obligated to be familiar with the set of rules and laws relevant to his or her research [34] and every researcher is personally responsible for the (ethically and legally) proper conduct of his or her research [35-36].

#### *3.3.1.1 The Helsinki Declaration*

The Helsinki Declaration was assembled in 1964 (most recent amendment in 2013 [37]) with modern history's examples of gravely unethical "medical" research in recent mind, and with the aims to never repeat such violations [38]. As an ethical codex (an assembly of rules) it is in not legally binding, but it still has a tremendous impact in the conduct of medical research and states that "No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration" (paragraph 10).

#### *3.3.1.2 Informed consent in register based studies*

Based on ethical principles such as Merton´s CUDOS* rules [39] from the 1940's and Beauchamp & Childress's "four principles of biomedical ethics" [32] from the 1980's**, the overarching goal of the declaration is to prevent any science from physically harming or otherwise violate the personal integrity of the study participants. It is logical that the concept of informed consent is central in the Helsinki Declaration.

The Helsinki Declaration covers all medical research, including register-based studies and studies on human material from bio-banks. In practice, a strict adherence to the principles of informed consent may not be realistic in large population-based register studies and the relevance of the Helsinki Declaration for such studies has been questioned [40-41]. In the current version of the declaration it is now stated that in "exceptional situations where consent would be impossible or impracticable to obtain…the research may be done only after consideration and approval of a research

ethics committee" (paragraph 32). This wording makes it possible to perform large register-based studies without informed consent from all included subjects, which would otherwise maker register-based research practically impossible.

Importantly, the basic principles of the Helsinki declaration is still highly relevant and applies to register-based studies as well. The potential violation of personal integrity of the participants in a register-based study must be carefully weighed against the scientific value of the study and the anticipated values for patients and society. Such evaluation of all studies involving humans is also mandatory according to Swedish law (Etikprövningslagen). The ethics committees in a sense, issuing an informed consent on behalf of the individuals in the register-based study that for practical reasons could not be obtained on an individual basis individually.

* ***Communism*** *(obligation to publish, common property) –* ***Universalism*** *(objectivity, peer review) –* ***Disinterestedness*** *(disclosure of interest) –* ***Organized Skepticism***

** ***Respect for autonomy****- the obligation to respect the decision making capacities of autonomous persons;* ***Non-maleficence****-the obligation to avoid causing harm;* ***Beneficence****-obligations to provide benefits and to balance benefits against risks;* ***Justice****-obligations of fairness in the distribution of benefits and risks*

## 3.4 DRUG SAFETY STUDIES

### 3.4.1 Study designs

*Analytic versus Descriptive studies*
According to a commonly used categorization of studies in medical science, studies are divided into descriptive or analytic. Descriptive studies can estimate specific features of individuals in relation to a certain outcome, i.e. who gets the disease, when and where. Analytic studies rank higher in the commonly referred hierarchy of study design [42], and can be divided into experimental or observational studies. Contrary to descriptive studies which are merely hypothesis-generating, analytic studies are designed to test a hypothesis about exposure-outcome relationships. This is achieved by the use of a comparison group.

Experimental versus Observational studies
Analytic studies are divided into experimental (e.g. randomized trials) and observational studies. The term "observational study" implies that the researchers observe individuals who receive a specific treatment or other exposure, but never actively allocate study participants to any of the treatment arms. Cohort studies and case-control studies are the main examples of analytic, observational studies. The cohort study design is commonly used when studying common and sometimes multiple outcomes, in a population where exposure is uncommon. If a fixed number of individuals are followed, the study is by definition a closed cohort. Cohort studies can also be defined as open, allowing for study participants to enter and leave the cohort during follow-up. The case-control design is preferably used to study rare outcomes, sometimes with multiple exposures

#### *3.4.1.1 Clinical trials*

The safety profile of a medicinal product (drug) is typically provided by clinical trials preceding market authorization, and post-authorization reporting of spontaneous adverse drug events [43-44]. These sources are valuable but associated with some shortcomings that can foil the attempt to provide a true safety profile of the drug. In clinical trials, adverse events are rarely the primary outcome, and the reporting of adverse events is often inadequate [44-45]. Also, the limited trial durations (typically 3-6 months) and the number individuals studied (typically less than 5000) make this study design unfit to detect adverse event that are uncommon, pharmacologically unexpected [46], or appear after a longer treatment period [47-49]. Another potential drawback is that the adverse events detected in the clinical trials program may have low generalizability to patients treated in clinical practice [44, 47-50]. Nevertheless, the experimental, and often blinded, setting reduces the impact of biasing factors such as confounding and channeling of sicker or healthier patients to any of the treatment groups. The use of randomized clinical trials with broader inclusion and exclusion criteria, so called "effectiveness" trials has been proposed as a complement to study the drug in a clinical setting [51-52].

#### *3.4.1.2 Spontaneous Adverse Event Reporting and Case Reports*

In the wake of the thalidomide disaster [53-54], post-market authorization routines and systems for spontaneous adverse event reporting from the health care professionals (and in some settings, from patients) has been implemented in Sweden and other parts of Europe, as well as in the United States and Canada [55-56]. Novel systems and collaboration for data-mining of medical charts for adverse events have been developed and evaluated [57-61].

Spontaneous reporting of adverse drug events may reflect the safety profile of the drug used in clinical practice [56] and to detect signals of uncommon, unexpected "idiosyncratic" type B, or "off-target" adverse events [46, 56, 62]. The system of spontaneous adverse drug events reporting is widely accessible to many "reporters" and has the potential to give a timely warning [43, 55, 62].

Another valuable contribution to the knowledge about the safety profile of a drug is reporting in the form of case reports as in thalidomide (phocomelia), [53] cerivastatin (rhabdomyolys), terfenadin (QT-prolongation), and troglitazone (liver failure) [63]. Apart from the problem of substantial underreporting (an underreporting of 95% in the US setting has been reported), a fundamental problem with case-report and spontaneous adverse drug event reporting is the lack of denominator. The number of treated individuals is unknown and therefore it cannot be readily determined whether the reported event occurs among 1% or 100% of individuals exposed to the drug.

#### *3.4.1.3 The cohort study in drug safety*

In 2011, a new legislation to strengthen all aspects of pharmacovigilance was introduced within the EU [58] and in 2012 EMA established the Pharmacovigilance and Risk Assessment Committee (PRAC) [64]. Examples of output from PRAC are advice and recommendations in risk management plans (RMP) and post authorization safety studies (PASS). The observational cohort study is a valuable tool in this perspective and in many circumstances it is the most rational choice of study design. Randomized clinical trials undoubtedly have the highest scientific ranking in the "hierarchy of study designs" [42] but they may be too administratively or economically challenging, unethical (e.g. studies of smoking or environmental pollutions), or otherwise unsuitable for reasons discussed above.

The observational cohort design allows the study of a large number of individuals over an extended period of time, with the possibility to investigate multiple outcomes. The principal shortcoming of the observational cohort study design is the lack of random allocation of treatment/exposure which may lead to confounding due to potentially uneven distribution of risk factors (for the outcome) at study start. It follows that causality cannot be established with certainty in an observational study. A well designed, well executed and well reported [65-66] cohort study can minimize the impact of lack of randomization. If data is prospectively collected and detailed information about important confounders is available, such a cohort study mimics the randomized controlled trial set up, but with better suitability for the follow-up of a large number of individuals treated in clinical practice over an extended time period. It must be recognized however, that unknown confounding can only be removed by well executed randomization of exposure.

## 3.5 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic disease of the joints characterized by persistent synovitis, joint destruction and systemic inflammation [67-68].

### 3.5.1 Etiology and risk factors

#### *3.5.1.1 Pathogenesis*

Despite the wealth of research in this field, a singular specific molecular pathway leading to the clinical presentation of RA has not been discovered. Rather, it is presumed that genetic, immunological and environmental factors interplay which result in the signs and symptoms that trigger the diagnosis [69-72]. The interaction between these factors may depend on circumstances such as disease phase (e.g. subclinical versus established RA) and disease subtype (ACPA positive versus negative), which could explain some of the heterogeneity in treatment response, clinical presentation and risk factors among individuals with RA [73].

RA is considered an autoimmune disease on account of its classical hallmark, the rheumatoid factor (RF). Little is known about to what (if any) extent RF contributes to the pathogenic mechanism of RA. Instead, more recently discovered auto-antibodies have entered the scene. Antibodies directed to citrullinated proteins (ACPAs) [73] show high specificity for RA, and most ACPA-positive RA are also RF-positive [68]. ACPAs are present several years before the onset of RA, and are strong predictors of disease progression supporting that ACPA may have a role in the pathogenesis of RA. Several mechanisms have been proposed [68, 73-75] including ACPA immune complexes formation and complement activation, triggering the immune system and the production of cytokines. Both celluar and humoral immunity are strongly implicated in the pathogenesis of RA. For example, it has been suggested that auto-reactive CD4+ T-cells are central in the "maturation" of the ACPA-antibodies and the transition from ACPA+ asymptomatic to ACPA+ symptomatic RA [72, 75-76]. B-cells may have several important functions in the initiation and development of RA, among those acting as antigen-presenting cells for T-cells [77]

#### *3.5.1.2 Genetic factors*

In a recent nested case-control study using Swedish register data and a large cohort of incident RA, it was concluded that 50% of the risk in ACPA-positive RA and 20% of the risk in ACPA-negative RA could be attributable to genetic factors [78]. There was no sex difference, but a stronger heritability in early-onset RA compared to late-onset. Other studies have suggested a somewhat higher genetic contribution to RA susceptibility [79]. Genome-wide surveys have identified multiple risk alleles associated with RA, most of them situated within the HLA locus. The strongest genetic risk is conferred by the HLA-DRB1 alleles, in particular those sharing a specific amino-acid sequence involved in the presentation of the antigen to the T-cells, the so called "shared epitope" [80].

#### *3.5.1.3 Smoking and other risk factors*

Since the first studies in the 1980's, smoking has emerged as the most important environmental risk factor in RA [70]. Smoking appears to be a risk factor especially in ACPA-positive disease and in the presence of the HLA-DRB1 shared epitope [81]. Smokers also have a worse prognosis [82]. It is hypothesized that compounds of the smoke may trigger self-immunity to citrullinated proteins in the lung, leading to the production of ACPAs [83]. However, the interaction between ACPA and smoking is less evident in other populations, and thus smoking may not be the only trigger of ACPA [69]. Other potential risk factors for RA include low alcohol intake [84] and obesity [85]. Hormonal factors (oestrogen levels) have also been investigated in this context [86]. Low levels of vitamin-D as well as air pollution have been proposed as risk factors, but recent studies found no firm evidence to support this [85, 87].

### 3.5.2 Diagnosis and epidemiology

#### *3.5.2.1 Classification criteria*

The diagnosis of RA is aided by widely accepted classification criteria, although such criteria were developed primarily to identify homogenous study groups in clinical trials. The criteria have evolved from the widely used 1987 ACR criteria [88], to the 2010 ACR /EULAR criteria [67]. The revised criteria emphasizes the detection of individuals with early stage disease, not to miss the postulated "treatment window of opportunity" 3-6 months after first RA symptoms. It has become evident that RA can be divided into two distinct phenotypes, based on the presence or absence of antibodies directed to citrullinated proteins (ACPA-antibodies) [75]. ACPA status is included in the revised diagnostic criteria [67].

#### *3.5.2.2 Prevalence*

As discussed above, the case definition of RA has changed over time, and studies of RA epidemiology will therefore differ accordingly in estimations of prevalence and incidence [89-90]. Most studies in Northern European or Unites States settings present prevalence estimates between 0.5-1%, from time-points using the 1987 ACR criteria [90-92]. A large register-based study from Sweden found an overall prevalence of RA of 0.77% (twice as high among females compared to males), corresponding to approximately 60,000 individuals in Sweden [90]. The highest prevalence is among 80+ (2,7% among females). The study did not reveal any association between geographical region (north-south/ east-west) and prevalence of RA, but lower education level was clearly associated with higher prevalence, particularly among individuals >60 years old.

#### *3.5.2.3 Incidence*

Estimates of RA incidence vary between 20 and 50 cases per 100,000 across Europe and the Unites States, but may be lower in Southern European countries [89, 93]. On account of different case definitions, the estimates of early RA vary greatly [89]

In Sweden, approximately 2,000 women and 1,000 males are diagnosed with RA annually, corresponding to an incidence of 56/100,000 person-years among females and 25/100,000 person-years among males (overall 41/100,000 person-years). The highest incidence is seen among 70-79 years of age, among both women and men [94].

### 3.5.3 Morbidity and mortality

#### *3.5.3.1 Comorbidity in RA*

Given its relatively high prevalence and its substantial contribution to morbidity and health costs, RA, along with other diagnoses within the overarching term

musculoskeletal disorders (MSD) is considered as an endemic disease [95-96]. Comorbidities found to occur more often in RA include cardiovascular disease, stroke, diabetes, infections and renal disease [97-103].

#### *3.5.3.2 Cancer in biologics-naïve RA*

The association between RA and malignancy has been the subject of study for several decades. Early RA cohorts with substantially heavier immuno-suppression detected an increased risk of cancer [104-106], but meta-analyses of contemporary studies indicate that overall rates of malignancies among patients with RA are not different than what is expected in the general population [107]. The unremarkable overall risk is however composed of site-specific differences. For example, there is a 2-3 fold increased risk of both lung cancer and lymphoma, present in early RA, [107-110] and a 20%-100% increased risk of non-melanoma skin cancer [108-109, 111-113] (see section 3.7.2)

#### *3.5.3.3 Mortality in RA*

In many settings [114] but not all [115], mortality rates in RA has continuously decreased since the 1960's. This may reflect the generally decreased mortality rates in the population, the improved management of RA, or both. Compared to the general population however, mortality in RA is still substantially increased [116-118]. Cardiovascular disease is the most common attributed cause of death, accounting for 30-50% of the excess death [114]. In a Swedish study, other dominant causes of death were due to respiratory (including pneumonitis), gastrointestinal, urogenital diseases and infection. Malignancy as a cause of death was not more common in RA than in the general population [116]. In a Finnish study evaluating cause of death among 1,000 individuals with RA followed from 1988-1999, hematopoietic malignancy (but not solid tumors) as cause of death was 2.5 times more common in RA compared to the general population [119]. In a Scottish inpatient cohort with a follow-up of 20 years, RA patients more often died from lung cancer and hematologic cancers, but less often from gastrointestinal malignancies, compared to national mortality rates [118].

## 3.6 TUMOR NECROSIS FACTOR (TNF)

### 3.6.1 TNF super-family and their receptors

Cytokines are short-lived messenger proteins which play critical roles in biologic processes such as cell growth, inflammation, immunity and cancer [120-122]. The cytokine tumor necrosis factor alpha (TNFα), also sometimes referred to as TNF, takes on a central role in orchestrating the immunological response to noxious stimuli, and TNF has a vital role in both innate and adaptive immunity [123]. There is also evidence of a central role for TNF in T-cell mediated cancer eradication [120, 124-125]. TNFß (lymphotoxin) is considered less biologically important and is less studied. TNFα is further referred to as TNF.

TNF belongs to a superfamily with 19, mostly transmembrane, proteins which are related to TNF. Apart from TNF, members of this family include lymphotoxin (TNFß), TNF-related apoptosis-inducing ligand (TRAIL) and receptor activator of NF-kB ligand (RANKL), and others [126-127]. Complementary to the TNF-superfamily ligands are their 30 receptor molecules, including TNFR1 and TNFR2- receptors [126] . TNF binds to its receptors TNFR1 and TNFR2, which are the primary targets for TNF-inhibitor drugs. The TNF-superfamily ligands are primarily expressed on activated immune-cells, while their receptors are broadly distributed o many cell types, including cancer cells. TNFR1 for example, is expressed on virtually every cell in the body [121].

### 3.6.2 TNF in the rheumatoid arthritis affected joint

Inflammation in RA mainly targets the synovial joints, which display an accumulation of inflammatory cells such as macrophages, T-cells, B-cells, neutrofiles, plasma-cells [128] (figure 1.). An overproduction of tumor necrosis factor (TNF) in the joint is the main driver of the synovial inflammation and bone erosion [129]. TNF is produced mainly by macrophages and T-cells in response to a self-fueling auto-immune process [75, 128, 130]. Almost all cells that are exposed to TNF activates the NF-kappaß pathway, which is the main trigger in TNF-induced inflammation [131] .

In addition to TNF, a number of other pro-inflammatory cytokines such as IL-6, IL-1 and granulocyte–macrophage colony-stimulating factor (GM-CSF) are locally produced. TNF is the key driver and regulator of the cytokine response [128, 130] and a most attractive cytokine target for drug therapy in RA and other chronic inflammatory diseases [129-130, 132].

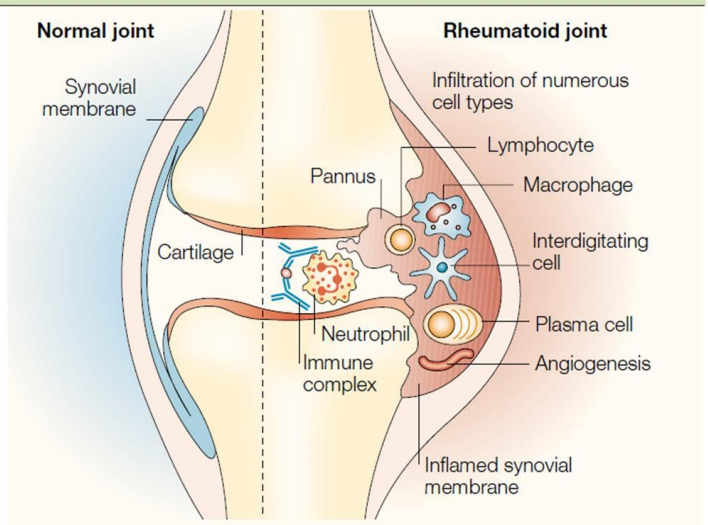


**Figure 1.** Comparison of normal and Rheumatoid joints

Adapted from Feldmann, M. Nat Rev Immunol 2002; 2 (5):364-71. Printed with permission

## 3.7 CANCER

Cancer development is ultimately the story of genetic alterations of a single cell, causing disturbed cell growth and cell cycle control and increased genetic instability [133]. Cancer is a cluster of heterogenous diseases and more than 100 cancer types have been described, each characterized by tissue origin and stage, and unique molecular signatures. Nevertheless, common major pathways in initiation, progression and spread may be affected. Tumor evolution involves either inherited genetic predisposition and/or DNA injury as a response to cellular stress. This contributes to a selection pressure towards unrestricted cell proliferation and accumulation of further mutations, and eventually the accumulation of a cancerous mass [133-134]. Apart from intra-cellular defense mechanisms, local cell-cell interactions and interplay with the immune system are important strategies to control tumor development [135].

**T**umor size, lymph **N**ode engagement and **M**etastasis are acknowledged prognostic factors for cancer, described through the TNM-classification system [24-25]. Each of the three dimensions have subdivisions which results in several (for some tumor types >20) potential combinations of TNM. These combinations are further condensed into clinical stages 0-IV, where 0 represents cancer in situ, and IV represents distant metastases[136]. Each clinical stage represents the same anticipated survival across tumor types, e.g. a patient with a stage 0 will probably survive the cancer, be it a colon, skin or breast cancer.

#### *3.7.1.1 Tumor suppressor genes, Oncogenes and signaling pathways*

One key feature in carcinogenesis is the acquisition of errors in cellular DNA (mutations), which ultimately lead to genetic instability and changes in cell growth control [137]. Such mutations or epigenetic changes of the cell DNA may be caused by endogenous factors (TNF has been postulated as one such factor, see section 3.7.3.2), or extrinsic factors like chemicals, radiation or viruses [134, 138]. Main targets for these genetic changes are the genes that normally control, cell growth, cell death and DNA-repair: proto-oncogenes, tumor-supressor genes and DNA repair genes [133, 137]. As a consequence tumor cells lose their self-limiting ability and exhibit either activated telomerase or a similar mechanism to maintain telomere length.

*Tumor-suppressor genes*

p53 is a tumor-suppressor which has been implicated in at least 50% of human cancers [134]. The p53 protein, encoded by the *TP53* gene, is a transcription factor that regulates several genes active in DNA repair, metabolism, angiogenesis, cell cycle arrest and apoptosis [139-140]. In response to DNA damage wild type *TP53* activation initiates proteins which promote processes of cell cycle arrest and apoptosis. Functional mutations in *TP53* prevent appropriate cell cycle “break” which promotes

uncontrolled growth and genetic instability. *TP53* inactivation has been argued to be a late event in tumorigenesis in some tumor forms, and an early in others [133, 140].

Less than 5% of malignant melanomas carry *TP53* mutations. Instead, functional inactivation of p53 target genes and the pRb pathway (a major regulator of cell cycle control) may be affected [141].

Fifty percent of BCC lesions and >90% of SCC lesions are found to have functional UV-related *TP53* mutations, mainly caused by the UV-B component of sun irradiation [134, 142]. Further, mutations in the tumor-suppressor gene *PTCH* causes faulty *Hedgehog*-signaling, typical for BCC [143].

*BCRA1* and *BCRA2* are other tumor-suppressor genes with well known relevance in hereditary breast and ovarian cancer. Their proteins act as transcription factors aiding in the repair of double stranded DNA. It should be noted however, that breast cancer is a highly heterogeneous disease and mutation in at least 40 genes have been implicated in the pathogenesis of breast cancer [144]. Also in breast cancer *TP53* mutations play a significant role [145].

#### *3.7.1.2 Oncogenes and signaling pathway*

Whereas the mutated tumor-suppressor genes prevent appropriate cell growth control, mutations or over-expression of proto-oncogenes (=oncogenes) leads to constitutive activation of growth promoting pathways [146] .The ERBB2-receptor which is a member of the Epidermal Growth Factor family of trans-membrane tyrosine kinase receptors is coded by the oncogene *ERBB2*, or *HER2*. Over-expression of *HER2* is seen in 20-30% of all breast cancers, and is often associated with worse prognosis[147]. The oncogene *RAS* family of GTPases (downstream intracellular mediator of *ERBB2*-signaling) has been found in 30% of all human cancers [121, 133, 137], and has been implicated as a "switch" that may render TNF to display tumor promoting, instead of tumor-protecting, features [148]. The oncogene *C-MYC* is a downstream effector in various signaling pathways controlling cell growth, differentiation and apoptosis. Over-expression of *C-MYC* has been implicated in many human cancers, including cancer of the breast [144, 149]. A wealth of signaling pathways upstream or downstream of known tumor–suppressors and oncogenes have been described, such as the MAPK cascade (a signaling pathway downstream of *RAS*). Forty to 60% of malignant melanomas have a defective MAPK signaling pathway, which is pharmacologically targeted in several novel BRAF–kinase, and MEK-inhibitors [150]. TNF interplays with many of the signaling pathways implicated in cancer initiation and progress.

#### *3.7.1.3 Tumor progression and metastasis*

Different genetic signatures have been associated with tumor initiation versus tumor progression and metastasis [151-154]. Tumor metastasis involves that a subpopulation

of the tumor cell mass acquires the ability to migrate from the tumor mass, enter the blood, disseminate and survive in the circulation, and to proliferate at a distant site [133, 155]. Some genes seem vital to tumor spread and metastasis, without having any evident impact on tumor initiation [151, 153, 156]. In breast cancer for example, significantly reduced levels of mRNA expression of the metastasis suppressor genes *BRMS1* and *KISS1* [154] have been associated with metastatic human breast cancer cells, but up to 70 different genes with relevance to breast cancer progression and spread, have been identified [147].

### 3.7.2 Skin cancer

#### *3.7.2.1 Malignant melanoma*

Malignant melanoma of the skin (melanoma, ICD-7 190) originates from the pigment producing melanocytes in the epidermis [157]. The major histopatologic type is the superficially spreading (SSM), while nodular type (NM) accounts for approximately 20%, and the akrolentiginous type (ALM) for a smaller proportion [157]. Clinical outcome is first and foremost related to tumor thickness measured in mm according to Breslow. Other prognostic features are infiltration (Clark 1-V) and ulceration [157]. The major risk factor for melanoma is UV-radiation from sun exposure [157], where intermittent sun-exposure in early age has been proposed as a particular risk [158]. Immune-suppression is another important risk factor (see below).

Melanoma comprises 5% of diagnosed cancers in Sweden, which makes it the sixth most common cancer. Approximately 3,500 cases of invasive melanoma are diagnosed each year, equal among female and males (35/100,000 person-years) and with a median age around 60 [159]. There is a geographic variation in melanoma incidence within Sweden [160].

#### *3.7.2.2 Non-melanoma skin cancer*

Non-melanoma skin cancer (NMSC, ICD-191) originates from the most abundant epidermal cell type, the keratinocyte. Eighty percent of NMSC is comprised of basal cell cancer and the remaining part mostly of squamous cell cancer [161].

NMSC generally have low metastasis rate and mortality in NMSC is mainly due to SCC [161].The predominant risk factor for NMSC is UV- radiation from sun-exposure, in combination with fair skin which burns easily[161]. Immune-suppressive states such as in AIDS or after solid organ transplantation seems to be particularly associated with the development SCC but only to a lesser extent with the development of BCC [162-163]. Other risk factors, such as male sex, human papilloma virus (HPV), and smoking are validated risk factors for SCC but seem less significant for BCC, indicating that these two cancers have partly different biology [164].

BCC accounts for 40% of the approximately 100,000 cancers diagnosed annually in Sweden [159]. NMSC (exclusive of basal cell cancer) comprises 10% of cancer in Sweden, which makes it the most frequent cancer apart from BCC, breast cancer among females and prostate among males. Approximately 5,800 cases of SCC are diagnosed annually with a prominent male dominance and a median age around 75 [159]. The incidence of NMSC is increasing with 5% per year [165]. There is a substantial geographic variation in NMSC incidence within Sweden, with an approximate incidence rate of ranging from 25/100,000 person-years among males in Jämtland (the Northen inland), to 125/100,000 among males in Halland (the South West coast) [160].

#### *3.7.2.3 Immune-suppression and skin cancer*

Melanoma

Therapeutic immune suppression as in organ transplant patients has been linked to an increased risk of melanoma [166-167], as have states of immune-deficiency such as in HIV [168]. Most studies of melanoma have not observed any increased risk compared to the general population (see supplementary table 1), but there are exceptions [169]. An Australian cohort study of 459 rheumatoid arthritis patients treated with methotrexate before 1986 reported a threefold increased risk of melanoma compared with the general population [169]. The accumulated disease activity and the spectrum of non-biological disease modifying anti-rheumatic drug use may have been substantially different from other, more recent cohorts. Effect modification by exposure to ultraviolet light (higher in Australia than in northern Europe) or skin type may also play a role.

Non-melanoma skin cancer

Several coinciding risk factors for a potential increased risk of non-melanoma skin cancer in RA have been proposed: smoking, chronic inflammation, deregulation of the immune system, alterations in innate tumor surveillance and potential oncogenic properties of several immune-suppressive therapies [170-171]. For instance, organ transplantation has been associated with a 10-fould risk of basal cell cancer (BCC) [162] and a 50-200 increased risk of squamous cell cancer (SCC)[162, 172-174]. Different classes of drugs probably confers differential risks of NMSC in post-transplant patients, with higher risks by azathioprine, cyclophosphamide and prednisolone, but perhaps to a lesser extent by calcineurin* inhibitors such as cyclosporine and tacrolimus [163, 171, 175].

The immune-modulating strategies in RA, with methotrexate as the anchor drug, are milder compared to in the transplant setting (see section 3.8, drug treatement in RA), but may still increase the risk of NMSC. Studies on biologics-naïve patients from

different settings and time-points have indicated a 20-100% increased relative risk of NMSC compared to the general population [108-109, 111-113, 176-177]. Askling et al. investigated the risk of non-melanoma skin cancer in a prevalent national cohort of mainly biologics-naïve RA compared to the general population. The risk increased with follow-up time which may indicate a role of cumulated immune-suppressive drugs or inflammatory disease burden [112].

Autoimmunity and an inefficient immune system may go hand in hand [178]. Individuals with hereditary immune-deficiencies are prone to develop autoimmune diseases, often autoimmune cytopenias, but also RA [179]. In ageing, the immune-system becomes less effective which involves dysfunction of T-cells and B-cells. At the same time, the risk of autoimmune disease such as RA increases. This seemingly paradoxical co-existence of a both ineffective and hyperactive immune-system has been viewed as a physiologic attempt to balance and counterbalance an immune response gone awry [178-179]. The difficulty in separating the immune-dysfunction associated with RA *per se*, from the immune-suppressive effect of DMARDs in observational studies such as those referred above, is well recognized [171].

**A phosphatase involved in activating the T-cells of the immune system [180]*

### 3.7.3 TNF and Cancer

*TNF in a historical perspective*
In 1891, the unexpected recovery of a patient with persistent, recurrent sarcoma of the limb, lead the New York Surgeon Dr William B. Coley to an intriguing discovery. The man with the sarcoma had suffered a severe erysipelas infection which seemed to have triggered the shrinking of the tumor. Inspired by the regressing sarcoma and occasional case-reports in the literature, Coley conducted a series of experiments administering weekly injections of viable streptococcus-extract to patients with severe malignancies. The first case, a man with an extensive ulcerating lymphoma of the neck, responded to the treatment with a severe attack of erysipelas. The lymphoma promptly regressed and the patient remained disease free for 8 years [120, 181]. Over the next 50 years, Coley´s toxin (a mixture of Streptococcus Pyogenes and Serratia Marcescens) was administered by Coley and co-workers, with varying results in thousands of patients with different types and stages of malignancy [182]. Coley believed that the bacterial toxin itself destroyed the cancerous cells, sparing the normal tissue. In 1975 it was proven that it was not the bacterial toxin, but instead the release of small proteins (cytokines) that elicited the destruction of tumor cells [120]. Specifically, and proven years later, the Coley toxin activates the immune system by acting as agonists on several Toll-like receptors (TLRs) and nuclear factor-κB (NF-κB) signaling [120]. In 1975, the term tumour necrosis factor was coined, and in 1985 the human and mouse TNFα-genes were cloned.

#### *3.7.3.1 TNF as a tumor-protective factor*

As the name implies, TNF has a well known ability to induce necrosis to human tumor cell lines of different types and has been extensively investigated with the hope of

finding a cure for cancer [183]. Furthermore, TNF's role as a major mediator in tumor cell destruction through several possible pathways has been postulated (reviewed in [121]). Induction of necrosis in the tumor vasculature, apoptosis of tumor cells, and T-cell mediated tumor cell killing are some of the major theories, briefly presented here.

*Tumor vasculature necrosis*

In the 1980's, human TNF was found to induce tumor necrosis (most prominent in sarcomas), in animal studies if injected locally and in high concentrations [184-185]. The tumor necrosis was hemorrhagic and caused by destruction of the tumor vascular bed, and this discovery seemed promising for the eventual development of an anti-cancer therapy in humans. However, it soon became evident that TNF administered systemically had an extremely narrow therapeutic window, with high risk of endotoxin shock –like symptoms.

To mitigate these adverse events, clinical trials using isolated limb perfusion instead of systemic administration, were performed [120, 186]. TNF with the addition of mephalane and interferon, or doxorubicin, was given locally in the affected limb to patients with malignant melanoma or soft tissue sarcoma, which resulted in remarkable regression of the tumors (but no overall increased survival) [187-188]. The TNF-analogue tasonermin (Beromun®) was approved in 1999 for use in advanced soft tissue sarcoma. Since then, several new approaches for TNF-mediated anti-cancer therapy have been evaluated with the primary target being tumor vasculature, or to sensitize tumors to other treatments, e.g. radiation [189-190].

*Apoptosis*

Many of the ligands of the TNF-superfamiliy (see section 3.6.1) and their receptors share the ability of inducing apoptosis via a "death domain" on the receptor, and thus have an important role in the immune defense against cancer. The binding of TNF to TNFR1 is associated with two principally different signaling pathways, each of them depending on the cellular context [120-121]. One pathway results in apoptosis, which is important in tumor surveillance. The other, which is the default pathway, induces genes and cellular response associated with inflammation and cell survival, and therefore the apoptotic properties of TNF is weak under "normal" conditions. In combination with metabolic inhibitors (i.e mephalan, see above) however, the default pathway is blocked and signaling is channeled towards apoptosis.

*T-cell mediated killing*

In addition to the direct lytic effect mediated through the release of cytotoxic granulae, human NK-cells induces apoptotic killing of tumor cells by activation of several members of the TNF-superfamiliy ligand-receptors, including TNF. Antagonists of TNF fully inhibited this NK-cell mediated killing in vitro [126]. Furthermore, CD8+ T-

cells, with TNF as one of several mediators [126], recognize tumor antigen in the context of MHC class I molecules. Thereby they play a major role in tumor surveillance, in particular in the defense against "immunogenic tumors" (tumors eliciting an immune response) such as malignant melanomas [124, 191] .

#### *3.7.3.2 TNF as a tumor-promoting factor*

Chronic inflammation is tightly intertwined with many states of cancer, either as its cause or its end-result [131, 192]. Many malignant cells and host cells in their microenvironment constitutively produce a small amount of TNF [121]. Animal models show that TNF produced in this context enhances the promotion, growth and spread of many tumor types [131] by mechanisms including angiogenesis and increased transition to metastatic activity [121]. Further, TNF produced in states of chronic inflammation stimulate oncogene (e.g. *C-MYC*) and tumor-suppressor (e.g. *TP53)* mutations. Based on the discussion of TNF as a major tumor initiating and promoting cytokine in inflammation-related cancer, TNFi has been investigated in oncology [193-194].

Mice-models investigating carcinogenesis as a consequence of chronic inflammation have revealed a dual effect of cytokines [148]. Inflammation- dependent tumor formation and protective antitumor response driven by TNF and interferon (so called "cancer immunoediting") was found to coexist in the same tumor model. The authors conclude that there is a complex interaction between the tumors and the immune system, and that this interaction is not an "all-or nothing event". There can be multiple outcomes where the immune system may both promote and eliminate developing tumors and sculpt tumor immunogenicity, depending on factors such as tumor microenvironment, tumor cell type and temporal circumstances [148]. Figure 2 outlines the two sides of TNF in tumor biology.

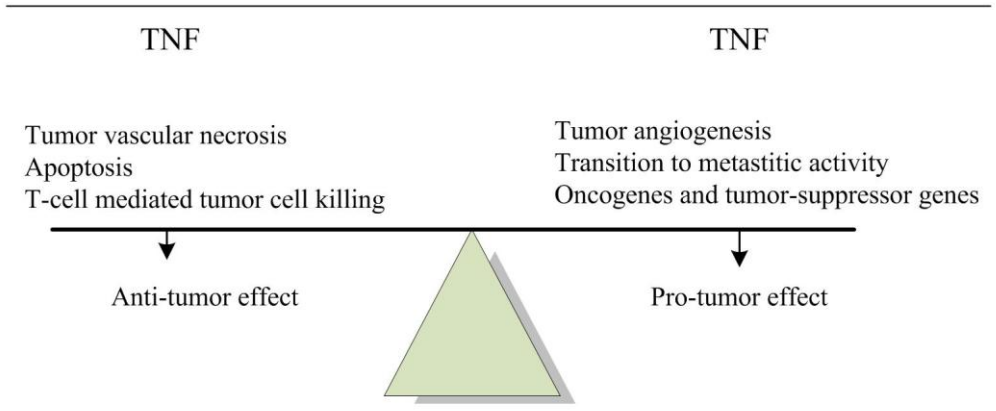


**Figure 2.** The dual effects of tumor necrosis factor (TNF) in tumor initiation and progress.

## 3.8 DRUG TREATMENT IN RA

### 3.8.1 General aspects and outline of treatment guidelines

The recent guidelines on pharmacological management of RA from the Swedish Society for Rheumatology (SRF)[195] are aligned to the EULAR 2013 new guidelines on drug treatment in RA [196]. Given the heterogeneous character of RA, the need for a differentiated and individualized treatment strategy, is stressed [196-197]. Overarching principles include that DMARDs should be initiated as soon as the RA diagnosis is made, remission or low disease activity should be the treatment target, and monitoring should be frequent (treat to target [198-199]). A rheumatologist should be primarily responsible for the treatment [196]. The following paragraphs describe some key messages of the SRF guidelines [195].

#### *3.8.1.1 Disease activity*

Choice of treatment strategy is largely dependent on RA disease activity, the occurrence of other factors associated with unfavorable prognosis (such as extra-articular manifestations and progressive erosions), and general health. The most commonly used clinical tools to ascertain disease activity are the 28 joint Disease Activity Score (DAS28), Simple Disease Activity Score (SDAI) and Clinical Disease Activity Score (CDAI). DAS28 includes the physician´s assessment of 28 joints, an objective inflammatory parameter (CRP or SR) and the patient's own assessment of his or her health status [200]. SDAI and CDAI are simplified versions of DAS28. Drug treatment of RA should aim to alleviate disease activity with the goal of achieving complete remission thereby halting progression into joint destruction and future disability. Criteria for remission have been defined [201].

#### *3.8.1.2 Conventional synthetic DMARDs*

Using the recently proposed new nomenclature for disease modifying drugs in RA, the traditional drugs such as methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, gold salts and others, are denoted conventional synthetic disease modifying antirheumatic drugs (csDMARDs) [202]. Methotrexate is the anchor DMARD in RA [195, 203]. It may be used as mono-therapy in individuals with low disease activity, as mono-therapy or in combination with other csDMARDs in moderate RA, or in combination with other csDMARDs or biologics in severe RA [195-196]. In patients presenting with low disease activity, mono-therapy with methotrexate or another csDMARD is recommended according to national guidelines [195]. In moderate disease activity, as a first step, methotrexate is the preferred drug in escalating doses up to 20-25mg/week, with evaluation of efficacy and tolerability after 2-3 months. Bridging corticosteroid therapy 5-7,5mg/week is recommended as concomitant therapy in the initial phase, complemented with intraarticular glucocorticoids therapy if needed. If this strategy fails, and the patient lacks

prognostically unfavorable symptoms and signs (see above), there is some evidence supporting combination therapy with methotrexate, sulfasalazine and/or hydroxychloroquine [195, 204-205]. The same csDMARD combination therapy with corticosteroid bridging can also be considered as first line treatment in RA presenting with high disease activity [195, 206-207].

#### *3.8.1.3 Biologics*

Biologic DMARDs (bDMARDs) include abatacept, adalimumab, anakinra, certolizumab pegol, etanercept, golimumab, infliximab, rituximab, tocilizumab, and biosimilars. In RA, TNFi treatment (see section 3.8.2) has so far been the first choice on account of the more extensive evidence of their efficacy and safety compared to the other bDMARDs [195, 208]. TNFi should be used in combination with methotrexate in order to enhance efficacy [209] and decrease the risk of neutralizing antibodies [210].

According to national guidelines [195], TNFi treatment in combination with methotrexate should be considered in individuals with moderate disease activity when methotrexate mono-therapy has failed. It is also a first line therapy in combination with methotrexate in RA presenting with high disease activity and several prognostically unfavorable disease characteristics (e.g. progressive erosions). This constitutes only a small fraction of the patients [196].

Among individuals with contraindications to TNFi, abatacept or tocilizumab should be considered [195]. Among individuals with contraindication to methotrexate, abatacept, tocilizumab or the three TNFi indicated for treatment without the combination with methotrexate (adalimumab, certolizumab pegol or etancercept) should be considered [195].

### 3.8.2 TNF-inhibitors

#### *3.8.2.1 Brief molecular structure and indications*

The European market currently holds five registered TNF inhibitors, listed here in type and alphabetical order: the three full-length antibodies adalimumab, golimumab and infliximab, the pegulated human fab-fragment certolizumab-pegol, and etanercept, a fusion protein of a TNF-receptor (TNFR2) extracellular region and the Fc fragment of the human IgG1 [211-215] (figure 3). All TNFi are approved for the treatment of adult patients with rheumatoid arthritis. Other indications, differential between the five substances, include juvenile idiopatic arthritis (JIA), psoriatic arthritis (PsA), ankylosing spondylitis (AS), psoriasis, Crohn's disease and ulcerative colitis[216-220] Through the blocking of TNF, TNFi have a multi-dimensional pharmacodynamic with effects on inflammation, tissue destruction and angiogenesis [128]

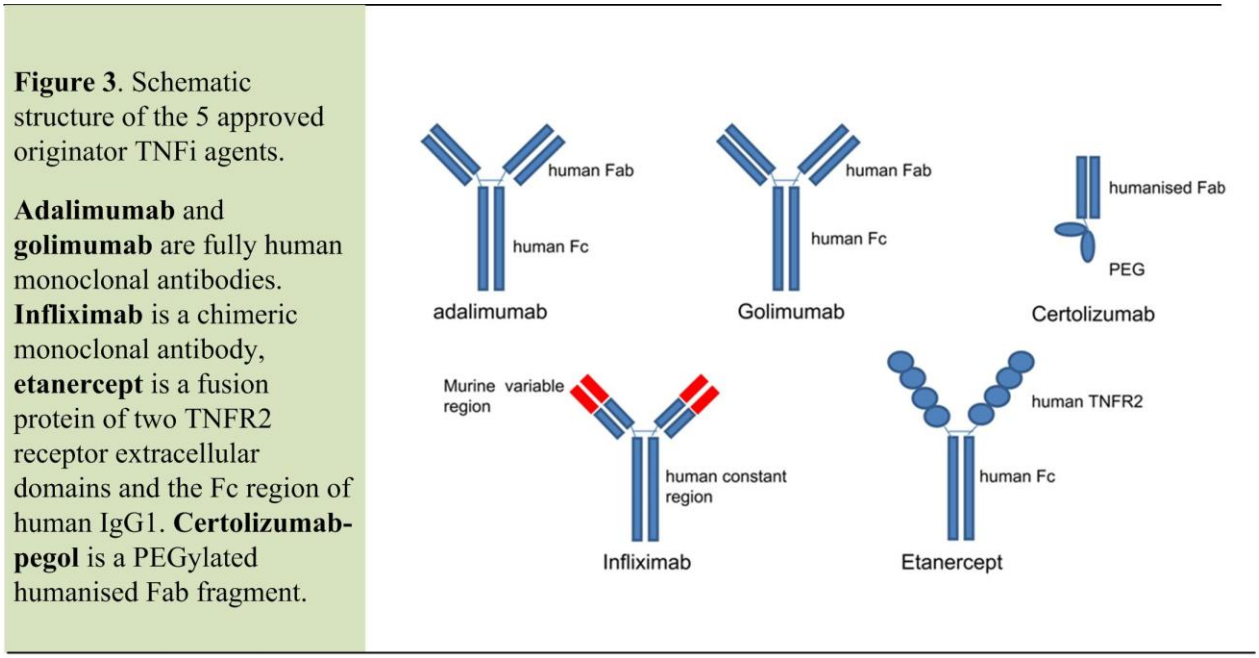


**Figure 3**. Schematic structure of the 5 approved originator TNFi agents.

**Adalimumab** and **golimumab** are fully human monoclonal antibodies. **Infliximab** is a chimeric monoclonal antibody, **etanercept** is a fusion protein of two TNFR2 receptor extracellular domains and the Fc region of human IgG1. **Certolizumab-pegol** is a PEGylated humanised Fab fragment.

Adapted from N. Thalayasingam, J.D. Isaacs / Best Practice & Research Clinical Rheumatology 25 (2011) 549–567. Printed with permission.

#### *3.8.2.2 Efficacy*

In reviews and meta-analyses of RCTs, all registered TNFi show similar efficacy in RA according to ACR50 [221] and similar safety, measured as withdrawals due to adverse events [222-225]. This evidence is based mainly on indirect (i.e. not head-to head) comparisons. The overall improvement according to ACR50 in the placebo comparisons is around 20%, compared to an overall effect of around 50% for TNFi. Overall, increasing doses did not improve efficacy. ACPA-status is a suggested, but not established, prognostic factor for treatment response in RA [226].

#### *3.8.2.3 Preclinical safety studies*

The preclinical trial programs of TNFi generally included studies of single and repeat dose toxicity with cardiovascular, respiratory and CNS endpoints (cynomolgus monkeys), genotoxicity, developmental toxicity and local tolerance. No major toxicological or genotoxic concerns were identified [211-215] . Carcinogenicity was not tested due to the lack of adequate models (no or low affinity for mouse/rat TNF). The lack of relevant pre-clinical studies on cancer risk as a potential safety issue is reflected in the risk management plans of TNFi, requiring post-marketing safety studies to assess this risk in clinical practice.

### 3.8.3 TNF inhibitors and cancer

Soon after introduction to the market, 26 cases of lymphoproliferative disorders which developed in association with TNFi treatment were detected in the FDA spontaneous drug reporting system [62]. Since then, malignancy associated with TNFi in RA has been evaluated in both clinical trials and meta-analyses of clinical trials, including

studies included in the market authorization holder´s risk management plans [227-231], and observational studies [110, 112-113, 176-177, 232-236], with somewhat inconclusive results.

#### *3.8.3.1 All-site cancer*

RCT-data

Short term risk of cancer was investigated in two meta-analyses of RA-patients receiving treatment during the first five years after the introduction of TNFi to the market. A threefold, and dose-dependent, increased risk of all-site cancer was observed in 3,500 RA-patients treated with adalimumab or infliximab, compared with 1,500 receiving placebo or csDMARDs [227]. A non-significant 80% increased risk was observed in 2,200 RA-patients treated with etanercept, compared to 1,000 receiving placebo or csDMARDs [228]. These findings raised concerns that TNFi treatment could induce rapidly growing tumors, or speed up the growth rate or otherwise alter the phenotype of pre-existing tumors. A later meta-analysis performed following a request by the EMA comprising 50% more RA-patients than prior assessments [229]. Including NMSC (for which there was a doubled risk) there was a 30%, near-significant, increased relative risk of all-site cancer among TNFi-treated compared to individuals receiving control. Differences in the meta-analysis approaches as well as differences among the included trials in terms of year of inclusion, RA-severity, csDMARD-treatment, and baseline risk of malignancy may contribute to the somewhat differential results in the meta-analyses [229]. Observational studies are needed as a complement for long-term follow-up of cancer in TNFi treatment.

Observational studies

Observational studies from the first decade following market authorization of TNFi [112-113, 237-238] and recent observational studies [177, 234, 239-240] indicate that TNFi-treated RA patients have no higher risk of all-site cancer than RA patients treated with csDMARDs (figure 4). Follow-up were considerably longer than the typical 6month-1year time-span of the clinical trials above.

Site-specific differences

When site-specific risk were assessed, TNFi-treatment conferred no increased risk of lymphoma lung, breast, prostate or colorectal cancer compared to a biologics-naïve RA comparator (which however had increased or decreased risks compared to the general population as described in section 3.5.3.2). Some signals of increased risk of melanoma [113, 177, 232] and NMSC however emerged [111, 113, 233, 241] (see below).

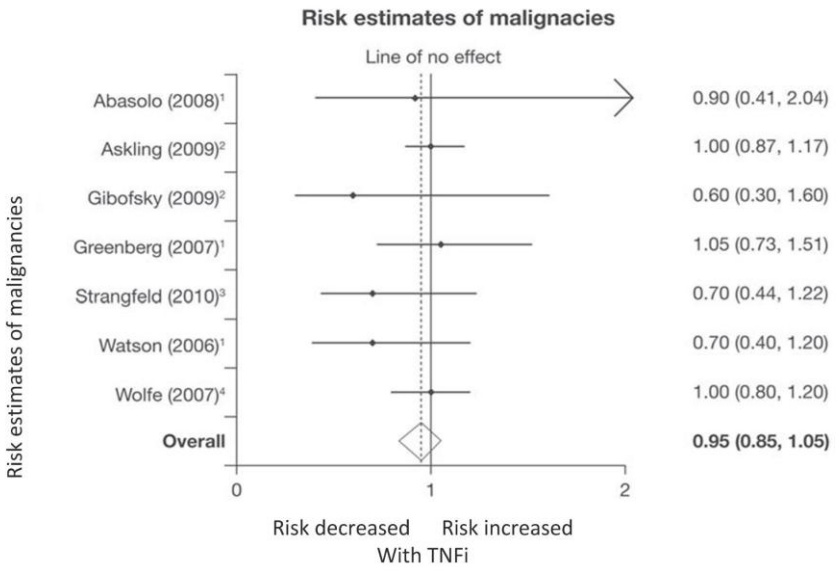


**Figure 4.** Risk estimates of all-site malignancies reported in prospective observational studies of rheumatoid arthritis patient treated with TNFi. Different measures of relative risk are displayed:1=IRR; 2=RR; 3=HR; 4=OR.

Adapted from Mariette, X. et al *Ann Rheum Dis* 2011;**70**:1895–1904. doi:10.1136/ard.2010.149419. Printed with permission

### *3.8.3.2 Melanoma*

Study I in this thesis was initiated in response to signals of increased risk of melanoma in TNFi-treated RA patients. These studies are briefly presented below.

RCT-data

In a recent pooled meta-analysis, estimates of risk for melanomas above one were observed for etanercept and infliximab but not for adalimumab, resulting in an overall odds ratio of 1.08 [230]. Based on only four melanomas observed in three randomized controlled trials of 52-104 weeks duration, the estimate had low statistical precision (95% confidence interval 0.1-10.2). Other meta-analyses have typically not reported specifically on risk of melanoma in association with TNF inhibitors [227-229, 231].

*Observational studies US/Canadian settings*

A cohort study using US and Canadian claims data investigated cancer risks in older rheumatoid arthritis patients exposed to methotrexate, biological drugs, or both [232]. The authors reported a doubled risk of melanoma among RA patients overall compared with the general population (standardized incidence ratio 2.3, 95%CI 1.6-3.2), but of the 29 identified melanomas only one occurred among biological-treated patients. A US community-based cohort study, including 13,001 patients with rheumatoid arthritis, of whom approximately 50% were ever treated with biological drugs, reported an increased risk of melanoma compared with the general population (standardized incidence ratio 1.7, 95%CI 1.3-2.2) [113] largely driven by melanomas among the TNFi-treated patients, with a relative risk of 2.3 (95%CI 0.9-5.4) comparing patients treated to not treated with biological drugs.

Observational studies European settings

A study from the Danish biologics register observed a potentially (statistically non-significant) increased risk of melanoma among TNF-treated (n=3,347, six melanomas) compared with non-biological drug treated RA patients (n=3,812, three melanomas; hazard ratio 1.54, 95%CI 0.37- 6.34) [177].

### *3.8.3.3 Non-melanoma skin cancer*

Study II in this thesis was initiated in response to signals of increased risk of melanoma in TNFi-treated RA patients. These studies are briefly presented below.

RCT-data

With respect to NMSC risks in patients starting TNFi treatment, a study including RCT data from 8,800 patients with RA detected no increased risk of NMSC among TNFi-treated (mean follow-up: 307 days), using either a meta-analysis approach (OR 1.27; 95%CI 0.67-2.42) or a pooled relative risk –approach (RR 1.41; 95%CI 0.41-4.91) [231]. On the other hand, a meta-analysis of 74 RCTs including more than 22,000 patients across a range of indications, mostly with trial durations of <6 months, showed an increased risk of NMSC among TNFi-treated RA [229]. The risk of NMSC (not distinguishing between SCC and BCC) was doubled among all TNFi-treated combined (HR 2.02 95% CI 1.11-3.95), compared to biologics-naïve comparators. Median follow-up of the included RCTs was 4 months; therefore the risk associated with longer follow-up could not be investigated.

Observational studies US/Canadian settings

Two studies using the US National Data Bank for Rheumatic Diseases (NDB)[111, 113] and one recent US study using administrative data [241], reporting relative risks of NMSC in TNFi-treated (versus biologics-naïve) RA ranging from 1.2-1.5. A meta-analysis of observational studies further supports a NMSC risk increase of the same magnitude [233].

Observational studies European settings

Studies in European settings have not confirmed an increased risk of NMSC associated with TNFi treatment in RA. In data from the Danish biologics register, 42 NMSC were detected among TNFi-treated and 34 among biologics-naïve, yielding a HR of 1.10 (95%CI 0.69 -1.76) [177]. SCC and BCC were included together as a composite endpoint, which may have diluted any true risk increase of SCC, if it exists. A recent study from the British biologics register investigated SCC and BCC separately [242]. The authors concluded that an increased risk of SCC could not be excluded, due to lack of power (23 SCC among TNFi-treated and 4 among biologics-naïve, HR 1.16; 95% CI 0.35-3.84). In the same study, no increased risk of BCC associated with TNFi treatment

was detected (150 BCC among TNFi-treated and 38 among biologics-naïve, HR 0.95; 95%CI 0.53 to 1.71).

#### *3.8.3.4 TNF inhibitors and cancer recurrence*

Study III in this thesis was initiated against the background of clinical guidelines advocating against the use of TNFi among RA-patients with a diagnosis of cancer within 5 or 10 years [243-244]. These recommendations rested mainly on experimental data (se section 3.7.3), but clinical data were scarce. Recurrent cancers of all type have been investigated in only two publications [240, 245]. In a study from the German biologics register (RABBIT) on cancer recurrence in patients with RA treated or not with biologics, with a follow up of 2.5 years, 9 and 5 recurrent cancers of different types were observed among 72 TNFi-treated and 43 biologics-naïve patients with a history of cancer. The corresponding HR for TNFi was 1.4; 95%CI 0.5-5.5. In a similar study from the British biologics register (BSRBR), with a follow-up of 3 years, 13 and 11 recurrences at any site were observed among 177 TNFi-treated and 117 biologics-naïve RA-patients with a history of any cancer, resulting in a HR for TNFi of 0.58 (95%CI 0.23-1.43). These studies were limited by lack of baseline data on cancer-related prognostic factors, i.e. any potential channelling bias could not be characterized. No prior study had specifically investigated recurrent breast cancer in RA-patients treated with TNFi.

#### *3.8.3.5 TNF inhibitors and post-cancer survival*

Study IV in this thesis was initiated in response to reports of rapid emergence of cancers soon after TNFi initiation [227-228] (see section 3.8.3.1), and the limited data on cancer prognosis among these patients. To my knowledge, apart from our study (IV), no publication has investigated post-cancer survival in TNFi-treated RA. In a US setting, mortality following cancer among patients with early inflammatory arthritis following cancer was increased with 40% compared to the local population [246], but the risk associated with TNFi treatment was not specified.

# 4 METHODS

## 4.1 STUDY DESIGN AND SETTING

In all studies in this thesis we used a population-based open cohort design with prospectively recorded data from national clinical-, health- and demographics-registers. We included individuals who fulfilled the eligibility criteria after a prespecified date, and followed them for the outcome of interest until a prespecified date (end of follow-up). The study participants were required to leave the cohort at the diagnosis of certain comorbidities (for example cancer other than the study outcomes), migration or death. For details on the use of cohort studies in drug safety, see section 3.4.1. We included individuals who fulfilled the eligibility criteria after a prespecified

date, and followed them for the outcome of interest until a prespecified date (end of follow-up). The study participants were required to leave the cohort at cancer other than the study outcome (study I) emigration or death. In study I and II, in order to increase efficiency, we used a matched design to estimate risks among biologics-naïve compared with the general population. Comparators were matched 5:1 to the biologics-naïve on age, sex, county and marital status. In study III and IV, in order to create balanced study populations at baseline, we used a matched design to estimate risks among TNFi-treated compared with biologics-naïve RA. Figure 5 illustrates the principles of the register linkages of the four studies.

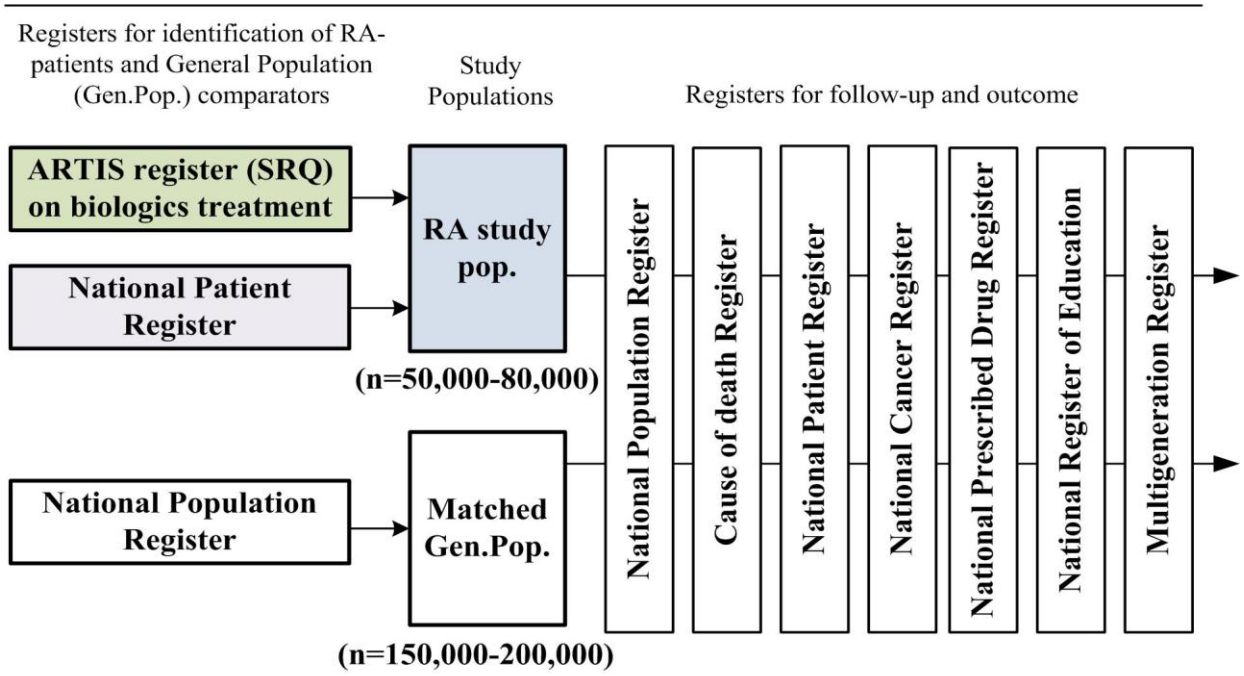


**Figure 5.** Principle of register linkages used to identify the study populations, follow-up and ouctomes. Adapted from Askling et al. Ann Rheum Dis 2006; 65(6):707.

### 4.1.1 Setting

The Swedish health care system is publicly funded which assures that health care provided for Swedish residents is not dependent on insurance or income status. This lead to small differences in access to care across geographic and socioeconomic strata. Patients with RA are typically managed by a rheumatologist working at hospitals rather than as private practitioners, with small regional differences in level of care.

### 4.1.2 Data Sources

#### *4.1.2.1 Data sources used to identify the study participants*

During the follow-up time-period of the four studies of this thesis, on average 70% of the biologics-naïve individuals were identified in SRQ, and only a few percent (<1% for the TNFi-treated) were identified in SRQ but not in the National Patient Register. We therefore identified our biologics-naïve study populations through the latter (see section 3.2.2), by using a strict definition of RA. This definition required at least two diagnoses with RA in the National Patient Register, at least one of them at a rheumatology or internal medicine unit. This method has proven to identify both incident and prevalent RA patients with high accuracy (*Kristin Widén, unpublished data*). The outpatient component of the National Patient Register was initiated in 2001, and hence Jan 1st 2001 was the earliest possible inclusion date for the biologics-naive individuals identified through this source (study I,II and III).

Among the individuals with RA identified through the National Patient Register, data on biologic treatment were collected in the ARTIS register of biologic treatment (see section 3.2.1). ARTIS includes individuals starting TNFi treatment from 1998 and onwards. Figure 6 illustrates the sources used to identify of our study populations.

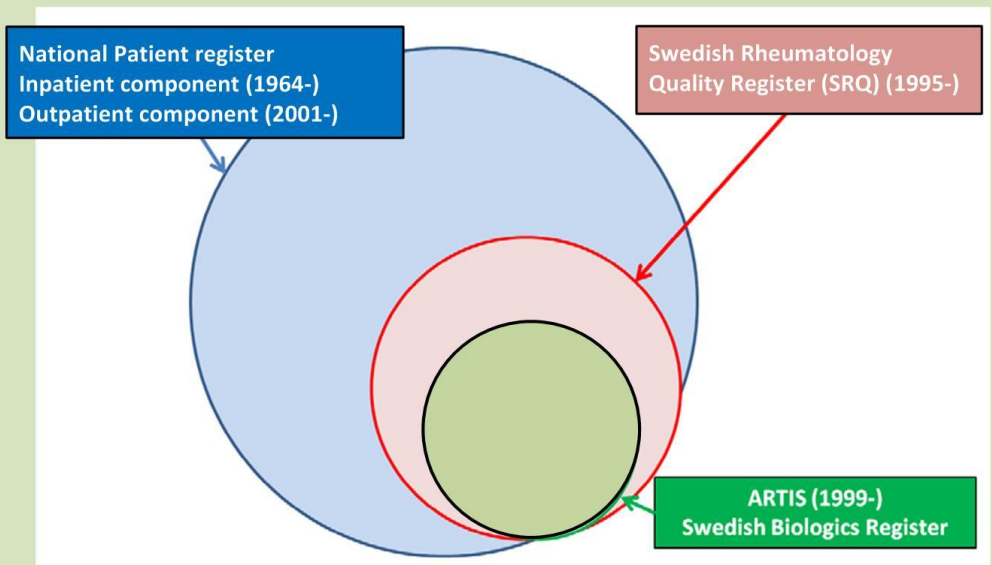


**Figure 6.**
Venn diagram showing the partly overlaping source populations for the identification of RA-patients in study I-IV.
Adapted from Neovius et al *Ann Rheum Dis* 2011;70:516–519. doi:10.1136 ard.2010.130914.
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#### *4.1.2.2 Data sources used to identify covariates and outcomes*

Study participants were followed up for outcomes using the National Cancer Register (study I-IV), the Cause of death Register (study IV) as well as data from medical files (study III) until the end of 2011 at the latest. BCC is available in the National Cancer Register since Jan 1st 2004, which served as start date for follow up for BCC in study II.

Information about covariates used to characterize the cohorts or adjust the analyses were collected from the nationwide quality of care, population and census registers and/or medical files from earliest 1958, and onwards. For an outline of the data sources used, see section 3.2. The different study end dates reflects the time-point of medical chart review for study III (Oct 1st 2011), and available register linkages at the time of data assembly for study I (Dec 31st 2010), II (Dec 31st 2011) and IV (March 31st 2009).

### 4.1.3 Paper I

#### *4.1.3.1 Rationale*

We hypothesized that the risk of melanoma could be increased following the immune-suppressive effects of TNFi treatment, since a competent immune response is important for the host protection of malignant melanoma**.**

#### *4.1.3.2 Design and subjects*

In this study we investigated the risk of malignant melanoma and all-site cancer in 11,343 TNFi-treated (1998-2010) and 49,136 biologics-naïve RA-patients, and in 204,054 matched general population comparators. See Supplementary figure 1 for flowchart of the study population.

#### *4.1.3.3 Exposure, outcome and follow-up*

We compared three exposure categories: biologics-naïve RA-patients, RA- patients starting a first ever treatment with any of the five TNF inhibitors approved in Sweden during the study period (adalimumab, certolizumab pegol, etanercept, golimumab, and infliximab), and the general population.

The primary outcome was defined as first invasive melanoma in individuals without any history of invasive cancer of any type. Secondary outcomes included *in situ* melanomas, second primary melanomas and all-site cancer. We followed the participants for outcomes and censoring (emigration, death or cancer other than the outcome) using national health registers until latest Dec 31st 2010.

#### *4.1.3.4 Potential confounders*

We adjusted for potential confounders prior to start of follow-up: country of birth, family history of melanoma, educational level, personal history of non-melanoma skin cancer in situ, hospital admissions/outpatient visits for knee/hip joint replacement surgery, chronic obstructive pulmonary disease, ischemic heart disease, and diabetes. To explore non-biological disease modifying anti-rheumatic drugs as a potential confounder, we used data from the prescribed drug register for the subset of our population followed from July 2005 through 2010.

#### *4.1.3.5 Sensitivity analyses*

To evaluate if different definitions of the biologics-naïve comparison cohort influenced the result, we performed sensitivity analyses using three sub-cohorts (incident RA, RA with stable use of methotrexate, and RA switching DMARDs) "nested" within the biologics-naïve cohort.

### 4.1.4 Paper II

#### *4.1.4.1 Rationale*

We hypothesized that the risk of NMSC could be increased in RA, and further in TNFi treatment, since immune-suppression in other diseases is a well recognized risk factor for NMSC**.**

#### *4.1.4.2 Design and Subjects*

In this study we investigated the risk of non squamous cell cancer (SCC) and basal cell cancer (BCC). We included 10,794 TNFi-treated RA-patients (1998-2011) for the SCC outcome and 7,397 TNFi-treated (2004-2011) for the BCC outcome. Similarily, we included 41,030 biologics-naïve RA-patients for the SCC outcome and 38,679 biologics-naïve for the BCC outcome, and matched general population comparators for each biologics-naïve cohort. See Supplementary figure 2 for flowchart of the study population.

#### *4.1.4.3 Exposure, outcome and follow-up*

We compared three exposure categories: non-biological drug treated rheumatoid arthritis patients, rheumatoid arthritis patients starting a first ever treatment with any of the five TNF inhibitors approved in Sweden during the study period (adalimumab, certolizumab pegol, etanercept, golimumab, and infliximab), and the general population.

The primary outcome included first *in situ* or invasive SCC or first BCC during follow-up while *in situ* and invasive skin cancers were evaluated separately as secondary

outcomes. We followed the participants for outcomes and censoring (emigration or death) through national health registers until Dec 31st 2011.

#### *4.1.4.4 Potential confounders*

We adjusted our main analyses for a series of potential confounders prior to start of follow-up: country of birth, educational level, marital status, county (proxy for sun-exposure) or history of the outcome (SCC or BCC). We also adjusted for comorbidities prior to start of follow-up (hospital admissions/outpatient visits for chronic obstructive pulmonary disease, ischemic heart disease, diabetes mellitus, knee/hip joint replacement surgery, psoriatic disease, any other diagnosis of benign skin disease except actinic keratosis) and use of immune-suppressive drugs prior to/during follow-up. We further adjusted for diagnosis of solid organ transplantation and invasive malignancy other than non-melanoma skin cancer, during follow-up.

#### *4.1.4.5 Sensitivity analyses*

We performed a series of sensitivity analyses by altering the definition of the study population, by altering the definition of the outcomes, and by altering the definition of biologics-naïve comparator.

*Comments study II*

A challenge with this paper was how to handle the presentation of the two outcomes SCC and BCC in a manner clear to the readers, but without overloading the text. Information on SCC is available in the cancer register from the start (1958), but information on BCC is available only since 2004 and onwards. This required us to define two separate study populations for SCC and BCC, with different start points of follow-up. This resulted in one study population for the investigation of SCC (n=41,125) and one for the investigation of BCC (n=38,751), both harvested from the same source population of biologics-naïve RA identified in the outpatient register 2001-2011(n=54,450).

Another issue worth mentioning is the potentially different ways of prioritizing between potential study outcomes. There are several alternatives that would be of scientific interest and that we had to consider. We contemplated with whether to split on invasive and in situ, or to use a composite of both. To study first ever, or first during follow up? Total burden of NMSC? Finally we agreed to define our primary outcomes as first invasive or in situ SCC or first BCC during follow-up, not excluding individuals with a history of NMSC prior to follow-up. This definition seemed closest to the routine clinical situation in which the rheumatologist considers to start TNFi or not, not always knowing the patients history of NMSC, and not primarily taking interest in the discrimination between risk of invasive or in situ. In order to help understand potential bias (primarily detection bias and reporting bias) we also thought it relevant to present HRs of invasive and in situ SCC separately. Finally we acknowledged the clinical importance and scientific interest of knowing the risk associated with TNFi treatment in a patient with a known history of NMSC. Therefore, we included this analysis as a sensitivity analysis.

### 4.1.5 Paper III

#### *4.1.5.1 Rationale*

We hypothesized that TNFi treatment in RA could increase the risk of recurrent breast cancer, since TNF has a vital but incompletely known relevance in tumor progression.

#### *4.1.5.2 Design and Subjects*

In this study we investigated the risk of breast cancer recurrence in RA treated with TNFi. All female TNFi-treated patients (1999-2010) with RA and a history of at least one diagnosis of breast cancer prior to the start of TNFi were identified through register linkages (n=143), and matched 1:1 from a cohort of 1598 biologics-naïve female RA-patients with a history of breast cancer. In patients with a history of more than one primary breast cancer, the latest served as index cancer.

The matching variables were sex, age at cancer diagnosis (±3 years), year of cancer diagnosis (±5years), cancer stage at diagnosis (invasive vs. *in situ*), and county of residence. One hundred and twenty TNFi-treated and 120 biologics-naïve patients met the eligibility criteria and were included in the final study population. See Supplementary figure 3 for flowchart of the study population.

#### *4.1.5.3 Exposure, outcome and follow-up*

We defined exposure as treatment with any of the five TNFi registered in Sweden during the study period. The primary outcome was first recurrence of breast cancer (relapse or new primary breast cancer). Through register-linkages and chart review of each individual´s RA- and breast cancer charts, we followed individuals for breast cancer recurrence (relapse or second primary) through October 2011.

#### *4.1.5.4 Potential confounders*

We adjusted our main analyses for a series of potential confounders prior to start of follow-up: RA disease severity and characteristics of the breast cancer at diagnosis (both described in detail below), education level, and hospital admissions/outpatient visits for chronic obstructive pulmonary disease, ischemic heart disease, diabetes mellitus, knee/hip joint replacement surgery.

Through the medical charts, we abstracted prognostic factors at breast cancer diagnosis, for breast cancer recurrence including tumor size (5 categories), nodal status (5 categories), distant metastases (yes/no), estrogen receptor status (yes/no), histologic grade (1-3, highest category =poorly differentiated cancer), as well as medical and surgical treatment (supplementary table 2 shows extraction form used for the clinical variables). We estimated RA disease activity during the 12 months period prior to start of follow-up and graded this as: inactive/low, moderate, or high. This categorization

was based on the clinicians' global assessment as noted in the records, and not primarily on formal disease activity scores. Information on conventional synthetic DMARDs (ever use), NSAID and/or oral steroids (regular use defined as > 4 consecutive weeks) was similarly abstracted.

#### *4.1.5.5 Information on clinical reasoning*

In addition to clinical data, we abstracted information on the physicians' decision to initiate TNFi (or not), which was coded in three categories among the TNFi-treated and four among the biologics-naïve patients.

#### *4.1.5.6 Web based risk prediction program Adjuvant!Online*

To further characterize any differential risk of recurrence between TNFi-treated and biologics-naïve at diagnosis beyond prognostic factors at index cancer diagnosis, we used Adjuvant!Online [247-250]. This risk model projects each individual patient's 10-year risk of relapse, or non- breast cancer death, largely derived from surveillance, epidemiology, and end-results (SEER) data and an overview from efficacy trials of adjuvant therapy [247, 251]. The reason for using Adjuvant!Online in our study was to characterize the two cohorts (with respect to recurrence risk) at the time of breast cancer diagnosis using an external, independent, and validated metric, rather than using the tool for an actual calculation of predicted recurrences.

### 4.1.6 Paper IV

#### *4.1.6.1 Rationale*

We hypothesized that TNFi treatment may have an impact on post-cancer survival, based on prior studies where rapid emergence of cancers after TNFi-initiation was indicated. Cancer stage at diagnosis could impact estimates of post-cancer survival.

#### *4.1.6.2 Design and Subjects*

We investigated the clinical stage at diagnosis and post-cancer survival, of cancers developing among 8,562 TNFi-treated (1999-2007), compared with 78,483 biologics-naïve RA-patients. We used an unmatched design, and matched design to account for cancer stage.

#### *4.1.6.3 Outcome, exposure and follow-up*

Study outcomes were defined as clinical stage at presentation of first primary cancers, and post-cancer survival. Exposure was defined as ever treatment with any of three TNFi treatments (adalimumab, etanercept, infliximab), or other biologics approved in Sweden during the study period.

For cancer stage at presentation, we compared the distribution of stage among the 302 cancers occurring among the biologics-treated to 586 cancers occurring among the biologics-naïve, using a matched design. Cancers were matched 1:2 for cancer site, sex, age (±5 years), and year of cancer diagnosis (±3 years). We used the information on TNM stage (coded into clinical stages 0-IV) available in the National Cancer Register, among the 302 TNFi-treated and the 586 matched biologics-naïve. TNM [24-25] classification is reported in the cancer register since 2003, and was available for around 30% of the cancers among the biologics-treated and the biologics-naïve. For each type of malignancy we created an algorithm to translate the TNM stage in the cancer register to a clinical stage (stage 0-IV), based on the established classifications available [252].

For post-cancer survival, we compared time to death of any cause following cancer among biologics-treated compared to biologics-naïve RA-patients, using both a matched and unmatched comparison. Individuals with a first primary cancer in the nationwide RA cohort were followed for outcome through register linkages until Dec 31st 2009.

#### *4.1.6.4 Potential confounders*

We adjusted our main analyses for a series of potential confounders prior to start of follow-up: the cumulative number of inpatient care episodes overall and for RA, and hospitalizations due to comorbid conditions (infection, ischemic heart disease, diabetes mellitus, COPD, or joint surgery). We also adjusted (using a stratified cox-regression model) some of the analyses for stage at diagnosis (see above).

#### *4.1.6.5 Chart reviews*

As a complement to the definition of stage trough the cancer register, we manually abstracted information from the medical charts for all TNFi-treated patients in whom breast, colorectal, lung, non-melanoma skin cancer, or prostate cancer was diagnosed between January 1, 1999 and December 31, 2005 (n= 86) and an equal number of biologics-naïve RA-patients who were individually matched for cancer site, year of cancer diagnosis, age, and sex. Through the medical charts we also assessed the validity of the RA and cancer diagnoses and the timing of initiation of TNFi treatment in relation to the occurrence of cancer.

## 4.2 STATISTICS

In cohort studies of adverse events (and several other types of studies and outcomes) the interest lies in a comparison of risk, or rates between two groups with different exposure-levels. Individuals in the compared groups may cease to be at risk of having the event of interest due to causes such as emigration (loss-to follow-up), death or the fact that the study ends. Such censoring must be accounted for in the different statistical techniques used in survival analysis [253].

### 4.2.1 Kaplan-Meier analysis

In survival analysis, the time to event can be estimated with the product limit or Kaplan-Meier method which produces an estimation of the survival function (survival probability and average survival time). The survival estimate is a probability and always a number in the interval [0-1]. In standard survival analysis such as Kaplan-Meier estimation, one important assumption is that censoring is independent; i.e. that the ones leaving the risk set due to censoring would have had the same risk of experiencing the event as the ones remaining in the risk set [254-255], which has implications for competing risks (see below). The non-parametrical logrank test is commonly used to compare differences between the survival functions associated with two different treatment groups. The logrank test does not, however, provide an estimation of the relative risk, and does not weigh in the impact of different prognostic factors that can differ among treatment groups, i.e. it cannot provide an adjusted estimate.

### 4.2.2 Cox Proportional Hazards Regression

The main statistical technique used in this thesis is the Cox proportional hazards regression model [256]. The Cox proportional hazards regression model is widely used in survival analysis, i.e. time-to event analysis [257]. The Cox model is often used to examine the effect of relevant prognostic values such as age, sex, weight, blood pressure, education or different treatments [258-259]. The model allows time-dependent covariates, i.e. prognostic factors that change over time [259-261].

The Cox model is comprised of a baseline hazard function, which may change arbitrarily over time and is not estimated by the model, and a set of covariates [258]. The hazard function describes the number of new events among individuals at risk per unit time. It can be thought of as the probability of instantaneous failure at time (t) given that the individual has survived up until (t)[258]. The model provides an estimate of the effects of the different variables entered into the model, and also estimates the relative hazard of experiencing an event, in an individual given its set of covariates (e.g. prognostic factors) [258]. An important caveat is the assumption of proportional hazards, which means that the ratio of the hazard of any two compared cohorts are proportional over time [259, 262-263]. Throughout the text I use the more

general wording “relative risk” to denote HRs and/or other relative measures of risk such as odds ratios, standardized incidence ratios or incidence rate ratios.

#### *4.2.2.1 Competing risks*

A competing risk is an event other than the study outcome which prevents the study outcome from occurring, or otherwise modifies the risk of the event of interest [255]. Death is a typical example of competing risk which is often highly relevant in studies of cancer related outcomes [264-265]. Discharge from the hospital in a study where the outcome is hospital infection, is another example [254]. In the interpretation of results from survival analysis, potential competing risks need to be considered [264, 266].

The Kaplan-Meier method yields biased results if there are more than one type of event (i.e. competing risks) and if these events are related, which generally can be assumed to be the case [267] . To handle the issue of non-informative censoring, the cumulative incidence proportion method can be used. Here, the competing risk is accounted for by treating it as an event, instead of censoring. The interpretation of the cumulative incidence proportion is that it estimates the risk of an event, given that individuals also can experience the competing risk. The cumulative incidence proportion does not reach 1. In study III we presented a cumulative incidence proportion curve to account for all-cause mortality as a competing risk. In study IV, the main outcome was all-cause death. All-cause death is robust to competing risks and the Kaplan-Meier curves should thus be accurate.

In many circumstances, the estimation of the HRs in a competing risk setting can be performed using a regular Cox proportional hazards regression (such as in the four studies of this thesis), then sometimes called “cause-specific hazard model” [255, 267]. It estimated the hazard of event in a setting where individuals also can progress to one or several competing events [264].

### 4.2.3 Statistics in the included papers

#### *4.2.3.1 General aspects*

We used the SAS software version 9.2 (SAS Institute, Cary, NC), for all analyses in studies I, II, III and IV. In addition to using SAS, we also used the R-package cmprsk for the calculation of the cumulative incidence proportion in the competing risk analyses for study III. In study I-III we tested the proportional hazards assumption (and found it not to be violated) by introducing an interaction term of exposure and log of follow-up time in the model. In study IV we assessed the proportional hazard assumptions by calculating HRs stratified by time since cancer diagnosis (<1 year, 1–4 years, or >5 years).

*Alternative time-scales*

In the main analyses of the four studies we used calendar time as time-scale in the Cox regression models (stratified also for year of inclusion into the study), to account for time-trends in cancer incidence and survival [159, 268]. We also evaluated the use of other time-scales such as follow-up time (stratified for year of inclusion), and attained age (stratified for birth year) to accommodate the difference in cancer risk among different ages [269]. These different model-specifications had minimal impact on the HRs, and thus we choose calendar time as the time-scale for our analyses.

*Alternative risk windows of exposure*

There are several potential definitions of "exposure" in terms of TNFi treatment. The adjudication of treatment-start is uncontroversial, but for how long the patient should be considered as exposed is not straightforward [66]. In study I-IV we used the "ever-exposed" approach, which is the most commonly used definition of exposure in observational studies of TNFi and cancer risk in RA [270]. Here, once the treatment has started (i.e.at least one dose given), the patient is considered at risk regardless intermittent or permanent treatment stop. In sensitivity analyses (study I and III) we redefined the risk window to include only the time-period when the individual was truly exposed, a so called "as treated" or "ondrug" (+ lag) approach. This was done by removing all follow-up time which fell outside the registered treatment periods+3 the months (arbitrary chosen to reflect the half-life and lingering pharmacodynamics). This alternative exposure-definition had minimal impact on the HRs (data not shown).

#### *4.2.3.2 Study I*

We used Cox regression to estimate hazard ratios, with calendar time as the timescale. TNFi treatment, comorbidities and drug use during follow-up were included as time-varying variables. In the analyses of TNFi-treated versus biologics-naïve RA, we adjusted hazard ratios for age at inclusion, sex, year of inclusion, and the potential confounders listed in section 4.1.3.4. Alternative timescales and model specifications yielded virtually identical results. We estimated hazard ratios overall and separately by age at start of follow-up, calendar period of starting TNFi, and time since start of first TNFi.

We used Cox regression to explore predictors of risk of melanoma within the TNFi-treated cohort. We assessed the following predictors at the start of treatment: age, sex, duration of RA, rheumatoid factor, and non-biological disease modifying anti-rheumatic drugs.

#### *4.2.3.3 Study II*

We used Cox regression to estimate hazard ratios, using calendar time as timescale. TNFi treatment, comorbidities and drug use during follow-up were coded as time-varying variables. In the analyses of TNFi-treated versus biologics-naïve RA-patients, the final, most adjusted model, was stratified for sex, year of inclusion, county, education level and civil status and adjusted for age at inclusion and a set of potential confounders including use of immune-suppressive drugs (se section 4.1.4.4).

We estimated hazard ratios overall and separately by sex, age at start of follow-up, calendar period of starting TNFi treatment, and time since start of first TNFi.

#### *4.2.3.4 Study III*

We used cumulative incidence curves to describe the probability of breast cancer recurrence, and all-cause death (to illustrate the potential that this was a competing risk). We used Cox regression to estimate hazard ratios (HRs). Biologics-naïve patients who started TNFi treatment (n=14) were censored at this time point, along with their matched TNFi-treated case. We performed a stratified Cox regression by age at diagnosis, year of diagnosis, county of residence and stage at diagnosis of index cancer and Cox-regressions adjusted for RA characteristics, comorbidities (listed in section 4.1.5.4), and characteristics of the breast cancer. We estimated HRs overall and separately by time since index breast cancer diagnosis at start of follow-up

#### *4.2.3.5 Study IV*

Tumor stage at diagnosis

Overall and site-specific distributions of stage were presented in a descriptive manner with p-values presented for selected strata.

Post-cancer survival-matched comparison

We compared post-cancer survival following the diagnosis of cancer among 302 biologics-treated and 586 matched (cancer site, sex, age (±5 years), and year of cancer diagnosis (±3 years) biologics-naïve RA-patients, using Kaplan- Meier curves. Cox proportional hazards regression analysis was used to calculate hazard ratios (HRs) of death following cancer, with the matched biologics-naïve group as the reference group.

Models stratified on the matching factors, and stage at cancer diagnosis and adjusted for age at cancer diagnosis as a linear term, cumulative number of inpatient care episodes overall and for RA, and hospitalizations with comorbidities (listed in section 4.1.6.4. were considered in models with alternative stratifications and adjustments yielded HRs similar to the less adjusted model, which was presented. This model was stratified for the matching factors and stage at cancer diagnosis (with missing stage as

one exposure level), with adjustment for age at cancer diagnosis and comorbid conditions. Models for site-specific survival were stratified for sex, age, and cancer stage only

*Nonmatched comparison*.

We compared post-cancer survival among 314 biologics-treated and 4,964 biologics-naïve RA patients. The Cox models were specified similarly to the matched analysis (see above). Separate analyses by age at cancer diagnosis (ages 16–49 years, 50–74 years, or ≥ 75 years), sex, year of cancer diagnosis (years 1999–2001, 2002–2004, or 2005–2007), cumulative duration of anti-TNF therapy (<1, 1–2, or >2 years, treatment status at cancer diagnosis (discontinued >6 months prior to cancer diagnosis or not), and rheumatoid factor seropositivity were also performed. Sensitivity analyses, including adjustment for comorbidity up until the diagnosis of cancer, were also performed, as were analyses that included only cancer cases for which the TNM stage was available.

# 5 RESULTS

## 5.1 PAPER I

Median follow-up was 4.8 years among the TNFi-treated and 4.6 years among the biologics-naïve. Thirty eight first invasive melanomas occurred in RA patients treated with TNFi; these patients had an increased risk of melanoma compared with RA-patients not treated with biological drugs (fully adjusted hazard ratio 1.5, 95%CI 1.0 - 2.2; 20 additional cases per 100 000 person years) (table 1).

One hundred and thirteen first invasive melanomas occurred in biologics-naïve RA-patients, and 393 occurred in the general population comparator cohort. Biologics-naïve RA-patients were not at significantly increased risk of melanoma compared with the general population (hazard ratio 1.2, 95% confidence interval 0.9 -1.5) (table2).

The risk of a second primary melanoma was non-significantly increased (hazard ratio 3.2, 0.8 -13.1; n=3 vs. n=10) in RA-patients treated with TNFi compared with those not treated with biological drugs.

Neither TNFi-treated (compared to the biologics-naïve) nor the biologics-naïve (compared to the general population), had any increased risk of first invasive all-site cancer (HR= 1.0; 95%CI 0.9-1-1. 1, table 1) and (HR= 1.1; 95%CI 1.1-1.2, table 2)

Using three different definitions of the biologics-naïve comparator resulted in the following hazard ratios for invasive melanoma among TNFi-treated compared with non-biological-treated RA-patients: First ever csDMARD initiators: 1.5 (0.8-2.9), “stable” methotrexate users 1.5 (1.0-2.4) , csDMARD “switchers”: 3.0 (1.2-7.6).

In the predictor analysis, neither the duration of rheumatoid arthritis nor concomitant use of non-biological disease modifying anti-rheumatic drugs at the start of the TNF inhibitor treatment emerged as predictors of melanoma.

**Table 1.** Occurrence and hazard ratios (HR) with 95% confidence intervals (CI), of cancer outcomes in 10,878 TNFi-treated patients with RA, compared with 42,198 biologics-naïve patients with RA.

| | TNFi (n events per person-years) | Biologics-naïve (n events per person-years) | HR[1] | HR[2] |
|---|---|---|---|---|
| Malignant melanoma | | | | |
| Invasive*¶ | 38/57,223 | 113/203,345 | 1.6 (1.1-2.5) | 1.5 (1.0-2.2) |
| In situ¶ | 11/56,080 | 57/197,754 | 1.1 (0.5-2.1) | - |
| All-site cancer | | | | |
| Invasive¶ | 558/55,947 | 2,788/196,826 | 1.0 (0.9-1.1) | 1.0 (0.9-1.1) |

***Primary outcome*
*¶ Among individuals without a history of any invasive cancer of any type*
*HR[1] Stratified for sex and adjusted for age*
*HR[2] Stratified for year of inclusion and adjusted for sex, age, country of birth, personal history of non melanoma skin cancer, family history of melanoma, education level and co-morbidities during follow-up (diabetes mellitus, ischemic heart disease, chronic obstructive pulmonary disease and joint surgery)*

**Table 2.** Occurrence and hazard ratios (HR) with 95% confidence intervals (CI), of cancer outcomes in 42,198 biologics-naïve patients with RA, compared with 162,743 matched general population comparators

| | Biologics-naïve RA (n events / person-years) | General population (n events / person-years) | HR[1] |
|---|---|---|---|
| Malignant melanoma | | | |
| Invasive*¶ | 113/203,345 | 393/854,111 | 1.2 (0.9-1.5) |
| In situ¶ | 57/197,754 | 219/838,548 | 1.2 (0.9-1.7) |
| All-site cancer | | | |
| Invasive¶ | 2,788/196,826 | 9,736/831,297 | 1.1 (1.1-1.2) |

***Primary outcome*
*¶ Among individuals without a history of any invasive cancer of any type*
*HR[1] Stratified for sex and adjusted for age*

## 5.2 PAPER II

Mean years of follow-up for the SCC analysis was 6.0 and 5.3 for TNFi-treated and biologics-naïve individuals with RA, respectively. As expected, follow-up was slightly shorter in the BCC study population

Comparing biologics-naïve RA to the general population, the HR of first *in situ* or invasive SCC in RA was 2.01 (95% CI 1.80-2.33). Similarly, comparing biologics-naïve RA to the general population, the HR of first BCC was 1.22 (95% CI 1.23-1.34).

Based on 168 vs. 803 first invasive or *in situ* SCC, the adjusted HR was 1.20 (95% CI 0.96-1.51) comparing TNFi-treated to biologics-naïve RA. The HR of SCC was driven mainly by *in situ* lesions. Based on 169 vs. 1,439 first BCC, the adjusted HR was 1.01 (95% CI 0.85-1.21) comparing TNFi-treated to biologics-naïve RA (table 3).

Including only individuals without a history of each of the outcomes before start of follow-up, the HRs for invasive or *in situ* SCC and for BCC were unaltered compared to the primary outcomes.

Analyzing lesions on the head/face and body separately, we detected a HR for invasive or *in situ* SCC of the head/face of 1.24 (0.98-1.58) and of the body of 1.1 (0.83-1.43) among TNFi –treated compared to biologics-naïve RA. The corresponding HR for BCC of the head/face was 1.2 (95%CI 0.9-1.7; 118 versus 1059 events) and of the body 0.9 (95%CI 0.6-1.4; ). Comparing the TNFi-treated cohort to three different subsets of the biologics-naïve cohort yielded the following relative risks of first invasive or *in situ* SCC: RA patients switching, or adding a DMARD: 1.3 (0.8-2.4), RA patients stable on methotrexate: 1.1 (0.8-1.5) and incident RA-patients: 1.4 (95%CI 1.0-1.9

**Table 3.** Occurrence and hazard ratios (HR) with 95% confidence intervals (CI), of squamous cell cancer (SCC) in 10,974 TNFi-treated, compared with 41,031biologics-naïve patients with RA. Occurrence and hazard ratios (HR) with 95% confidence intervals (CI), of basal cell cancer (BCC) in 7,397 TNFi-treated, compared with 38,679 biologics-naïve patients with RA.

| | TNFi (n events / person-years) | Biologics-naïve RA (n events / person-years) | HR[1] | HR[2] |
|---|---|---|---|---|
| Squamous cell cancer | | | | |
| First during follow-up | 168/ 66,010 | 803/221,081 | 1.24 (1.04-1.47) | 1.20 (0.96-1.51) |
| Invasive | 61/ 66,673 | 334/ 22,3571 | 1.12 (0.84-1.50) | 0.98 (0.71-1.35) |
| In situ | 126/ 66,224 | 580/222,080 | 1.25 (1.03–1.53) | 1.26 (1.02-1.57) |
| Basal cell cancer | | | | |
| First during follow-up | 169/ 29,432 | 1,439/184,441 | 1.14 (0.97-1.36) | 1.01 (0.85-1.21) |

**HR[1]** *Stratified for sex, county and civil status. Adjusted for age*
***HR[2]*** *Stratified for sex, county, civil status and education level. Adjusted for age, country of birth, history of the outcome (SCC or BCC) before follow-up, co-morbidities before/during follow-up (hospital admissions/outpatient visits for chronic obstructive pulmonary disease, ischemic heart disease, diabetes mellitus, knee/hip joint replacement surgery, psoriatic disease, any other diagnosis of benign skin disease except actinic keratosis), drug use before/during follow-up (ever use of cyclosporine, cyclophosphamide or azathioprine) and diagnosis of solid organ transplantation and invasive malignancy during follow-up.*

## 5.3 PAPER III

The median time from breast cancer diagnosis until TNFi treatment/start of follow-up was 9.4 years. As expected, TNFi-treated patients had more severe RA. Biologics-naïve patients were more likely to have lymph node engagement and were more often treated with mastectomy and chemotherapy at diagnosis of their breast cancer. The predicted 10-year risk of recurrence using Adjuvant!Online risk score and counting from diagnosis of the breast cancer was 18% among the TNFi-treated and 19% among the biologics-naïve (Supplementary table 3).

During a total of 592 person-years of follow-up among the TNFi -treated patients, 9 patients developed a breast cancer recurrence compared with 9 recurrences during 550 person-years of follow-up among the matched biologics-naïve patients. Comparing TNFi-treated to biologics-naïve patients, the HR for recurrence was 0.8 (95%CI 0.3-2.1). Adjusting for nodal status, type of surgery and chemotherapy at index cancer, the HR was 1.1 (95%CI 0.4-2.8, table 4 and figure 7).

**Table 4**. Occurrence and hazard ratios of recurrent breast cancer in 120 biologics-naïve and 120 TNFi-treated individuals with rheumatoid arthritis.

| | Biologics-naïve n=120 | TNFi-treated n=120 | |
|---|---|---|---|
| Total person-years of follow-up | 550 | 592 | |
| Individuals with recurrent breast cancer | 9 | 9 | |
| Rate /1000 person years (95% CI) | 16 (7-31) | 15 (7-29) | |
| | | HR* | HR** |
| Hazard ratio of recurrent cancer (95% CI) | 1 (reference) | 0.8 (0.3-2.1) | 1.1 (0.4-2.8) |

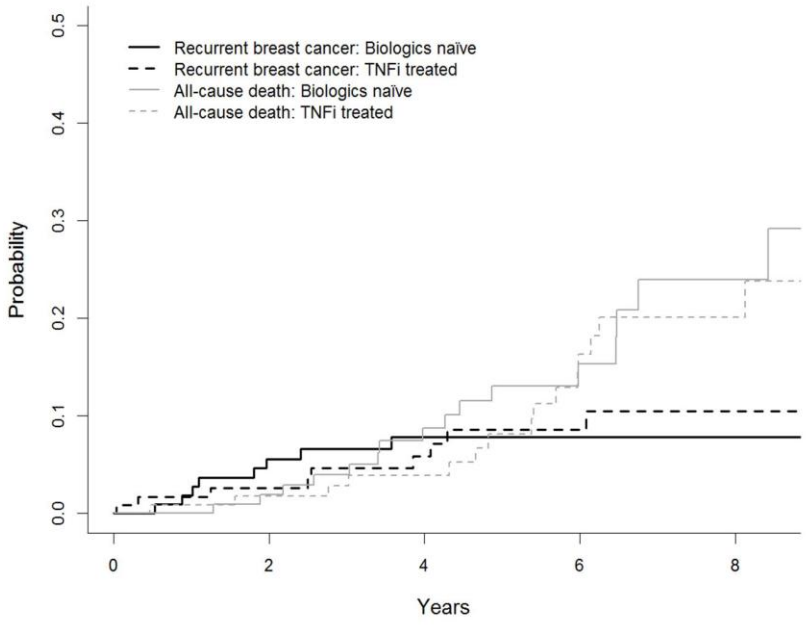
** Hazard ratio, adjusted for the matching factors*

*** Adjusted for breast cancer characteristics (nodal state, type of surgery, chemo-therapy) and comorbidities (diabetes mellitus, ischemic heart disease, chronic obstructive pulmonary disease and joint surgery).*

When stratified by time between the breast cancer and TNFi- initiation, the HR for recurrence among patients who started TNFi within five years from their breast cancer was 1.4 (95%CI 0.2-8.6) and 0.8 (95%CI 0.3-2.4) among patients who started TNFi more than five years after their breast cancer (p for difference =0.6).

The cumulative incidence of all-cause death was approximately 30% among both TNFi-treated and biologics-naïve during follow-up. All died from causes unrelated to breast cancer (figure 7).

**Figure 7.** Recurrent breast cancer and all cause mortality in 120 TNFi-treated individuals and 120 matched biologics-naïve individuals with rheumatoid arthritis and a history of breast cancer.



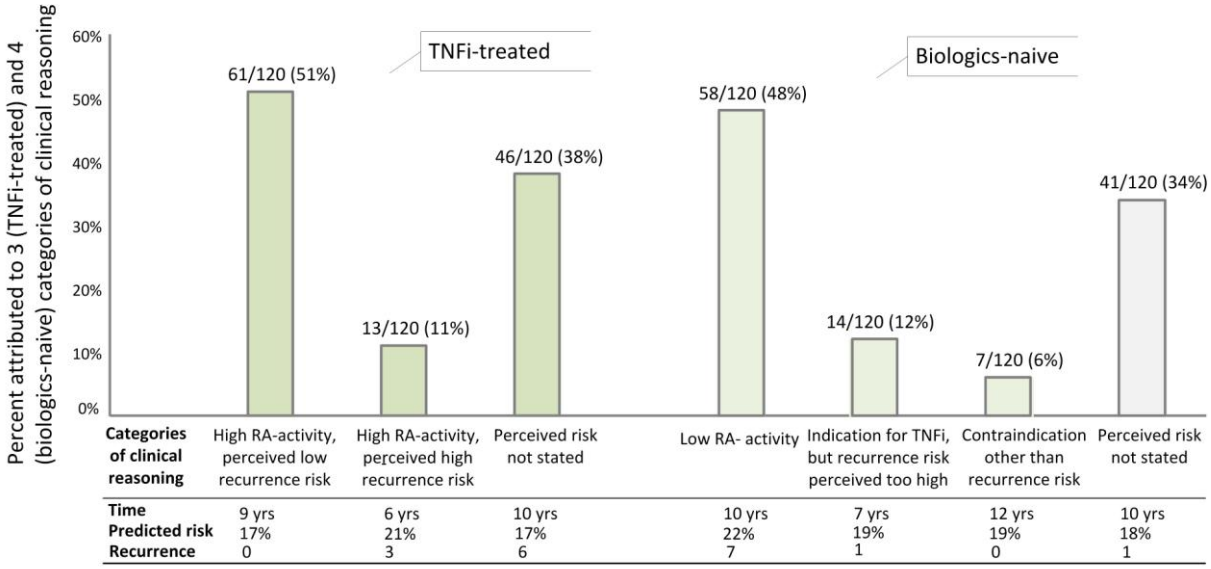



**Figure 8.** Physicians reasoning in the context of initiating TNFi or not, in 120 TNFi-treated and 120 biologics-naïve patients with rheumatoid arthritis and a history of breast cancer. Time=time between diagnosis of index breast cancer and start of follow-up; Predicted risk=predicted (Adjuvant!Online) risk of recurrence (IQR)%; Recurrence=number of recurrences during follow-up.

Figure 8 summarizes the clinical reasoning in relation to TNFi treatment. Thirteen individuals (11%) among the TNFi-treated initiated TNFi due to a compelling clinical indication although the recurrence risk was considered substantial. Conversely, 14 (12%) among the biologics-naïve did not start TNFi due to a perceived high risk of recurrent breast cancer, even though there was clear indication for the therapy .

## 5.4 PAPER IV

Tumor stage at diagnosis.

For all cancers combined, the distribution of stage at cancer diagnosis was largely similar comparing the biologics-exposed and the matched biologics-naïve RA-patients.

Post-cancer survival rates.

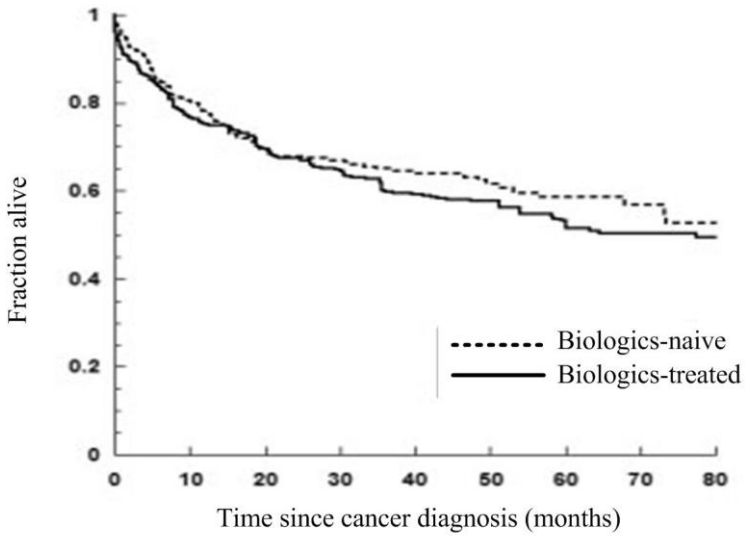
All except 2 (anakinra) individuals in the biologics-treated group were exposed to TNFi as first biologic drug. Among the biologics exposed RA-patients in which cancers occurred, the mean post-cancer follow-up time was 4 years. Among all of the 4,650 cancer cases occurring in the biologics-naïve cohort, mean follow-up time was 5 years.

Matched comparison.

Based on the total of 113 deaths among those with cancer in the biologics-treated group versus the 256 deaths among those with cancer in the matched biologics-naïve group, the relative risk of death following cancer associated with TNFi treatment was 1.1 (95% confidence interval 0.8–1.6) when accounting for the matching factors and TNM stage

(figure 9). None of the site-specific HRs indicated any statistically significant association between TNFi treatment and cancer survival. Further adjustments for comorbid conditions up until the start of anti-TNF, as well as sensitivity analyses including only cases in which information on the cancer stage was available, altered the HR less than 10% (data not shown).

**Figure 9.** Kaplan-Meier curve corresponding to risk of death following cancer occurring in RA-patients who were receiving or who had previously received therapy with biologic agents and in matched RA-patients who had never received such therapy




| No. at risk | | | | | |
|---|---|---|---|---|---|
| Biologcs-naive | 586 | 342 | 198 | 103 | 45 |
| Biologiscs-treated | 302 | 173 | 89 | 38 | 11 |

Unmatched comparison.

Comparing survival among the 314 biologics-exposed cancer cases to that of all 4,650 cases of first primary cancers occurring in the biologics-naïve RA comparator group, the HR was 1.0 (95% CI 0.8–1.3), taking the matching factors and stage into account (Table 5).

Sensitivity analyses including only cases for which information on cancer stage was available resulted in a similar result (HR 1.0; 95% CI 0.6–1.6). Similarly, none of the analyses stratified by sex, age at cancer diagnosis, rheumatoid factor, biologics agents discontinued >6 months prior to cancer diagnosis (or at diagnosis), time since biologics-start, cumulative duration of biologics indicated any difference in HRs across strata (p >0.3 for difference across strata for each comparison). Analyses restricted to deaths for which cancer was listed as the underlying cause of the death yielded similar results (HR 1.0; 95% CI 0.6–1.5). Similar to the matched comparison, none of the site-

specific HRs indicated any statistically significant association between TNFi treatment and cancer survival.

**Table 5.** Unmatched comparison: deaths following cancer diagnosis among 4,964 incident first primary cancers occurring in a national cohort of 78,483 patients with RA, of whom 8,562 patients were treated with biologic during 1999–2007: 314 cancers occurred among biologics-treated patients, and the remaining 4,650 cancers occurred among the biologics-naïve

| | Cancers in biologics-treated RA-patients (n=314) | | Cancers in biologics-naive RA-patients (n=4,650) | | Adjusted HR (95% CI)* for death following cancer |
|---|---|---|---|---|---|
| Cancer site | No. of cases | No. of patients who died | No. of cases | No. of patients who died | |
| All sites combined | 314 | 113 | 4,650 | 2,666 | 1.0 (0.8-1.3) |
| Breast | 48 | 8 | 655 | 209 | 1.0 (0.5-2.0) |
| Lung | 39 | 30 | 438 | 394 | 0.9 (0.6-1.3) |
| Colorectal | 26 | 13 | 438 | 271 | 0.9 (0.5-1.6) |
| Prostate | 21 | 2 | 656 | 238 | 0.5 (0.1-2.2) |
| Malignant melanoma | 22 | 3 | 141 | 57 | 1.2 (0.4-4.2) |
| All hematologic | 38 | 17 | 460 | 299 | 0.7 (0.4-1.3) |
| All other sites | 120 | 40 | 1862 | 1,198 | 0.8 (0.6-1.3) |

*Of the 314 cancers occurring in rheumatoid arthritis (RA) patients treated with biologic agents, 312 were in TNFi-treated, and 2 were in those taking other biologic agents (both anakinra).*
*† Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were determined by Cox proportional hazards regression, stratified for age, sex, type of cancer, and stage at cancer diagnosis (tumor-node-metastasis stage of cancers for which information was available, and adjusted for year of cancer diagnosis.*

*Rheumatology and Oncology medical file reviews*.

As expected, patients in the biologics-exposed group had evidence of more severe RA; for example, more of them had erosive disease (94% versus 59%), a history of>3 DMARDs (69% versus 32%), and corticosteroid use for >4 consecutive weeks (70% versus 55%)

When stage at cancer diagnosis was defined according to the information in the medical files (as opposed to the TNM coding in the Swedish Cancer Register), there was no statistically significant difference in the distribution of stage (localized/regional spread/distant metastases) between the two groups (54%/35%/11% versus 52%/28%/19%).There was no difference between the two groups with respect to the proportion of cancers diagnosed through patient-reported signs/symptoms versus through investigations primarily performed for other purposes.

# 6 GENERAL DISCUSSION

## 6.1 METHODOLOGICAL CONSIDERATIONS

### 6.1.1 Limitations and strengths

The four studies have a few limitations in common. We lacked information on potentially important confounders such as disease activity and RF/ACPA-status for a substantial proportion of the biologics-naïve individuals. A full history of csDMARD exposure was not available among the majority of TNFi-treated and biologics-naïve, since the RA-diagnosis was often prevalent at the time of inclusion into the registers. We lacked information on smoking. We had on average 5 years of follow-up (maximum around 12 years) which may be insufficient for an adequate description of cancer incidence and post-cancer survival.

The main common strengths of the studies were the use of a population-based design with prospectively recorded data. This ensured low misclassification of data on exposure, confounders and outcome, and negligible loss to follow-up. The Swedish Cancer Registry differentiates between *in situ* and invasive malignancies, and provides TNM-stage of tumors reported in 2004 and later. Specific strengths and limitation are discussed for each study in section 6.2.

### 6.1.2 Bias and Confounding

#### *6.1.2.1 Accuracy*

In any study, experimental or observational, there is a possibility that the results do not reflect the truth, in the sense that they convey an "inaccurate" or flawed conclusion. Such a study is said to have low accuracy, which in turn is a concept that commonly includes both precision (random error) and validity (systematic error) [271]. Study precision may be viewed as the opposite of random errors (sampling variability)[271]. A larger sample size increases precision, and precision can also be enhanced by modifying the study design [271].

#### *6.1.2.2 Statistical testing and confidence intervals*

Statistical testing is used in order to make inference about the measurement of disease association in the source population using estimates from a sample of the population [272]. A probability level (α-level) is arbitrarily set, often to 0.05. This implies that we have less than 5% probability of stating a difference among the study groups, which is not really true (less than 5% risk of rejecting a true null-hypothesis). A confidence interval gives a range of possible size estimates with a given confidence level. The corresponding confidence level to an α-level of 0, 05 is 95%, which is the confidence interval used for the presentation of the HRs in study I-IV. The interpretation of a 95% confidence interval is that if the test was (infinitely) repeated the confidence interval

would contain the true estimate in 95% of the times [271-272]. If the confidence interval of a HR does not include 1 there is a statistically significant difference between the between the comparison groups (usually exposed and unexposed individuals).

It must be recognized however, that any statistically significant result must also be viewed in the light of the potential existence of a plausible biologic hypothesis, the correctness of the statistical model as well as in the light of potential random error and/or systematic error.

In study I and II, our main findings of elevated risks of melanoma and non-melanoma skin cancer were statistically significant at the level mentioned above. For some of the stratified analyses, precision was limited and those results must be interpreted with caution.

#### *6.1.2.3 Statistical power*

Statistical power is related to the number of study participants needed to detect a certain pre-specified difference between the study arms with some probability (often 80%) and a given precision.

In study III, we lacked power to conclude that the null result (no significant difference between TNFi-treated and biologics-naïve) was "true", in a sense that there would still be no (clinically relevant) difference even with a larger study size. Based on the narrow confidence intervals of the unmatched comparison of post-cancer survival, and to a lesser extent for the matched comparison, in study IV, we conclude that this study had sufficient power to demonstrate a lack of (clinically relevant) difference between the compared groups.

#### *6.1.2.4 Validity*

A study has high *internal* validity when there are no biases distorting the association in the study population, compared to the "true" association in the study source [271, 273]. *Externa*l validity, or generalizability, refers to how relevant these estimations are for an extended population other than the one under study. The major threats of internal validity can be classified (although this classification is not always clear-cut) into any of the following three categories: selection bias, information bias, and confounding [271, 273]. Systematic errors are indifferent to sample size, implying that increasing the sample size will not mitigate the consequences of bias.

#### *6.1.2.5 Selection bias*

When selection bias is present, the relation between exposure and outcome is different among individuals selected for participation (study population), and the underlying population from which those individuals were sampled (study source) [271, 273]. In

some situations the concept of selection bias and confounding (see section 6.1.2.7) overlap.

*Disease severity*
Selection by disease severity, sometimes called channelling bias or confounding by disease severity, is a potential source of bias in all four studies of this thesis, as we compare the relative risk of cancer incidence or overall survival among biologics-naïve RA compared to TNFi-treated RA. RA-patients starting TNFi treatment suffer from active disease where csDMARDs are contraindicated or have failed, and are thus more severely ill than the RA patients who remain biologics-naïve. A more severe RA infers increased inflammation and higher burden of prior and concomitant csDMARDs, and possibly increased general frailty and risk of specific comorbidities [97-103]. Such patients my suffer an increased risk of cancer or reduced post-cancer survival. We were not able to adjust our analyses for such "disease severity". RA-specific variables such as RA duration, ACPA/RF-status, DAS-28, HAQ and full history of csDMARD-treatment were available only for a subset of individuals (mainly TNFi-treated).

Instead, in study I and II we performed sensitivity analyses restricting the comparator (biologics-naïve) to subsets which experience the same particular "selection forces" [271] as our TNFi-treated, i.e. high inflammatory activity or otherwise unstable disease as in DMARD "switchers". We also used two other clinically recognizable sub-cohorts of the biologics-naïve to study the impact of choice of comparator (incident RA without longstanding disease, and RA stable on methotrexate). None of the analyses using any of the three definitions of the biologics-naïve comparator revealed any major impact on the relative risk of melanoma or SCC associated with TNFi. Our interpretation based on those sensitivity analyses is that no particular distribution of potential confounders (unless common to all the three sub-cohorts) or factor associated with the therapeutic context of starting a new drug regime, is a major driver of our results.

### *6.1.2.6 Information bias*

Incorrect measuring of continuous variables (measurement error) or failure to classify a discrete variable correctly (misclassification) are examples of information bias, which can be differential or non-differential. Differential misclassification occurs when the misclassification of a variable is depending on a second variable (e.g. if classification of an outcome depends on exposure status) [273].

All data in the study I-IV was registered prospectively in a clinical context, and in several separate national health and administrative registers with high coverage. This reduces the potential for misclassification. Nevertheless, data collection in a register is never perfect. The RA diagnosis, TNFi treatment, outcomes and confounders used in the four papers of this study could potentially be subject to, sometimes simultaneously, misclassifications. Manual reviews of the medical files were performed among 172

TNFi-treated and matched biologics-naïve individuals who were diagnosed with solid cancer in study IV (see section 4.1.6.5), a subset of the TNF-treated melanomas in study I, and among all individuals in study III (see section 4.1.5.4). Partly, the rational for these reviews were to describe the amount of misclassification of RA, TNFi treatment and cancer.

*Missclassification of the Rheumatoid Arthritis diagnosis*

In study IV, we identified our study population through the inpatient (virtually complete) and outpatient register (90% coverage for RA). Chart reviews based on high retrieval rates confirmed the RA diagnosis in 96% of the biologics-naïve individuals in study IV which indicates that misclassification of RA was low. Similarly, a validation study of 800 individuals captured in the inpatient register 1964-1994 with RA as primary or secondary diagnosis, observed that 90% of the diagnoses were correct according to the 1987 ACR RA criteria [88, 274].

In studies I and II, we used a stricter definition of RA in the biologics-naïve population. We required minimum two or more separate visits with RA as primary or secondary diagnosis, and one of these visits had to be at a department of rheumatology or internal medicine. A recent validation study confirmed the high correctness of the RA diagnosis among individuals captured in the outpatient register using such strict criteria. 91% of the biologics-naïve RA had a verified RA diagnosis according to the 1987 ACR and/or 2010 ACR/EULAR criteria, and the remaining patients had other rheumatic disorders (*Kristin Widén Unpublished data*). To further minimize misclassification of RA in all four studies, we excluded individuals with any of the following diagnoses: AS, JIA, PsA and SLE.

In study III, the medical charts were scrutinized on a case-by-case level and individuals who were found not to have RA were excluded (only 2 cases, 1 osteoarthritis and 1 SLE among the 139 TNFi-treated and 139 matched biologics-naïve) ( supplementary figure 3).

Missclassification of TNFi treatment

A recent validation study supports a low rate of misclassification of TNFi treatment [275] . Coverage of TNFi treatment in ARTIS vis-à-vis the national prescribed drug register was estimated to 95%, and the proportion of individuals registered with TNFi treatment in ARTIS, who did not fill a prescription within 180 days was less than 2%.

Missclassification of outcome (cancer and death)

In all studies, we used the National Cancer Register for data on outcome. Coverage of the Cause of death Register is virtually complete [9]. Coverage of the National Cancer Register is around 95% overall, but varies depending on cancer site (for breast cancer,

coverage is almost complete, but for skin cancer a validation from 1998 indicated near 10% missing [23]. Any misclassification is likely non-differential, i.e. not dependent on exposure status.

In study III, we excluded individuals where the breast cancer could not be confirmed in the medical charts (only 1 case among the 139 TNFi-treated and 139 matched biologics-naïve) (supplementary figure 3). In study IV, chart reviews in a subset (n=172) confirmed the cancer diagnoses (breast, colorectal, lung, non-melanoma skin cancer, or prostate cancer) in all cases.

Protopathic bias

Protopathic bias represents a form of reverse causality. This bias arises when early signs of the outcome are the cause of initiation of treatment/exposure [276-277]. One illustration would be if first sign or symptoms of cancer are mistaken for RA or exacerbation of RA. In this respect, it represents a differential misclassification of exposure in relation to timing of the outcome [277]. Similarly, if the TNFi start date in ARTIS does not match the true treatment start, this could lead to a differential misclassification of the outcome (cancers diagnosed early after ARTIS start date may have occurred before the treatment was actually commenced, but detected due to pre-treatment screening or work-up). A recent validation study indicated that median lag time between ARTIS start date and a filled prescription was 3 days, and thus the window of opportunity for misclassification of exposure/outcome has to be considered narrow. The medical charts review in study I confirmed that all of the 20 reviewed melanoma antedated the TNFi start. In study III, one recurrent cancer was detected as lung metastases only 2 weeks after TNFi treatment initiation which may be an example of protopathic bias. Excluding this individual from the analysis did not alter the HR in any significant way (whether keeping the matched biologics-naïve comparator in the data set, or not). In study IV, 93% of the reviewed cancers were truly incident, but in 6/86 cases (7%) the first recorded sign or symptom of cancer was actually evident in the medical files prior to TNFi start.

In summary, chart reviews of study I, III and IV, revealed that misclassification was not a major issue, neither of RA, nor of TNFi treatment or outcome in the studies of this thesis.

Detection bias

The risk of adverse events is often heightened during the early treatment phase [278]. In the context where a new drug is initiated, the patient is subjected to intensified clinical, radiologic or laboratory examinations, i.e. increased surveillance. Apart from a genuine pharmacologic effect, increased detection may thus have an impact on that risk. (On the other hand, contraindications to TNFi and the potential for detection of

cancer through pre-treatment investigations before starting a TNF inhibitor might have led to a selection of patients with an a priori lower risk of cancer).

For all main outcomes in our studies there is a potential for differential risk depending on time since treatment start. The overall HRs may thus be misleading [278-279]. Whether HRs varied with follow-up was evaluated in studies I, II and IV by estimating HRs stratified for follow-up time (≤1yr; >1-5 yrs; >5 yrs). In study I, we detected no apparent difference between the stratified HRs for melanoma and thus no particular indication of detection bias associated with treatment start. The same finding has previously been demonstrated for all-type cancer in a study partly using the same study population [238]. In study II, the HR of SCC was moderately increased during the first 5 years of follow-up, but not increased thereafter. This may indicate a selection of low-risk individuals, a "depletion of susceptibles", rather than a decreased risk after 5 years [280]. In studies III and IV, Kaplan-Meier curves and incidence proportion curves were used as a complement to the overall HRs, with no indication of differences in relative risks depending on follow-up time.

TNFi-treated may have higher chance of having an adverse event detected not only during the initial phase, but during the full treatment course. We evaluated this through the medical charts (study I, III and IV), but found little evidence of systematic surveillance bias. For example, in study III we observed little difference in cancer stage at diagnosis among TNFi-treated and biologics-naïve. Post-cancer survival will inevitably be linked to how early the cancer was detected, which could introduce a so-called lead-time bias in studies of cancer survival [281-283]. Earlier detection would prolong the time between diagnosis and death, but not necessarily by increasing the individual´s life-span. To adequately evaluate post-cancer survival we therefore needed to account for cancer stage at presentation.

#### *6.1.2.7 Confounding*

Confounding, which is described as a "confusion" of effects [271, 284] is an undesirable element in analytic studies. It describes an association between exposure and outcome, which is not necessarily false, but which may irrelevant for the causal effect of the exposure on the outcome [273, 285]. An uneven selection (between exposure groups) of individuals with particular risk factors for the outcome may create confounding, and this is the Achilles heel of the observational study. A random allocation, randomization, of exposure is the most effective way of minimizing confounding. In observational studies randomization is not an option and only known and measurable confounding can be controlled for. This could be done in the study design by restriction (as in studies I and II) or by matching on the confounding variables (as in studies III and IV), or in the analyses of data by stratification or adjustment (as in studies I-IV).

*General information about confounders in study I-IV*

In the four studies of this thesis we chose potential confounders that were previously known or biologically plausible, and which fulfilled the criteria for a confounder (associated with the exposure and the outcome, without being on the causal pathway). Contrasting the biologics-naïve RA against the general population comparator in study I and II, we adjusted the analyses only for age, sex, education-level but not comorbidities, which may have arisen after the onset of RA (i.e. the exposure).

Studies III and IV were matched cohort studies. The matched analysis of post-cancer survival in study IV was adjusted for the matching factors in addition to other confounders [286]. In study III we were able to adjust either for the matching factors or for other confounders, due to power constraints. Whenever a more complex model yielded virtually the same HRs as a less adjusted model, the latter was presented in the published studies. Generally we had little indication of the existence of particularly strong confounders. Most of the variables used in the final models, apart from age and sex, altered the HRs by less than 10% when introduced in a stepwise matter (see section 4.1 for outline of specific confounders in each study).

*Source of confounders*

We adjusted for a variety of confounders including demographics, education, and comorbidities (such as history of malignancy and transplantation, and medications). Information on most confounders, except for those in study III, were retrieved from the national health and census registers described in section 3.2. We thus considered misclassification of these cofounders a limited problem.

*Age and Sex*

Many medical conditions and the propensity of receiving treatment have strong, associations with age and sex, which makes it necessary to somehow control for these variables. We adjusted all studies for age at start of follow-up as a linear variable which, for all outcomes, translated to an increased risk of around 3,5 % per increased year. Using age in 5-year intervals as stratification variable in the Cox regressions resulted in the same alteration in the HR of outcome. In studies I, II and IV, the multivariable analyses were stratified by sex. Study IV included only female individuals.

*Comorbidities*

A set of comorbidities and joint surgery were defined as a proxy for general frailty in studies I-IV. By adjusting for these comorbidities, we limited the impact from factors associated with a generally worse health status, and (or) multiple doctor´s visits (i.e. detection bias). In study I we adjusted the relative risks of melanoma for diagnosis of melanoma among first degree relatives, since family history of melanoma is a known

risk factor [287]. A history of cancer is a well known risk factor for a new malignancy [171, 288-289] and also impact the likelihood of receiving TNFi. Patients with a malignancy (including NMSC) prior to inclusion were excluded from study I and II, and invasive cancer during follow-up was adjusted for. Organ transplantation is a particularly strong risk factor for NMSC (see section 3.7.2) and such patients were excluded from study II (and organ transplantation during follow-up was adjusted for). UV-radiation is a well recognized risk factor for skin cancer, in particular for SCC. There are some geographical differences in TNFi penetrance [10] and potential differences in UV-exposure depending on residency, with a typically higher solar irradiation in coastal and southern parts of Sweden [290]. We adjusted our analyses of SCC and BCC in study II, for 21 geographical regions graded according to the sum of the annual CIE-weighted (a scale to mimic the erythemal effect of UV radiation) sun irradiation 1999-2011 for each region [290].

By default, we assessed comorbidities up until start of follow-up. Sensitivity analyses in studies I-IV assessing comorbidities up until diagnosis of cancer yielded essentially the same HRs.

Education

We adjusted all main analyses for education level. RA incidence [291] and disease severity (and possibly the propensity of receiving TNFi) is associated with socioeconomic status inclusive of smoking [82]. Regardless the relative egalitarian Swedish society there are also differences in risk of site-specific cancer incidence [159], and life-expectancy [292] depending on education level. Individuals with middle or high (upper-secondary or post-secondary) education level have an approximately 5 years longer life-year expectancy beyond the age of 30, compared with individuals with low (compulsory) education level [292]. Incidence of melanoma and NMSC is positively associated with higher educational level, possibly related to life-style factors including sun-exposure and attitudes toward health screening procedures.

Non-biologic concomitant medications

Similar to the well recognized risk of skin cancer in patients receiving potent immune-suppression following organ transplantation, csDMARDs have been postulated as a risk factor (see section 3.7.2). Against this background we used the Prescribed Drug register to adjust the estimations of melanoma risk (study I) for methotrexate exposure and, the estimations of SCC and BCC (study II) for ever use of azathioprine, cyclosporine or cyclophosphamide. Among TNFi-starters in SRQ-ARTIS, we observed that concomitant methotrexate, versus other csDMARDs, was not a predictor for melanoma (although power constraints limited firm conclusions).

*Comments study I*
One issue that we carefully considered during the process of our work was the potentially confounding effects of csDMARDs (especially methotrexate) on our findings of increased melanoma risk among TNFi-treated. To act as a confounder, csDMARD-exposure would need to be associated with TNFi treatment. Our chart validation in study III and IV have indeed confirmed that a higher proportion of TNFi-starters had been exposed to ≥ 3csDMARDs, compared to those who remained biologics-naïve and we may presume that the burden of methotrexate is generally higher among TNFi-treated. It is however important to emphasize that in most cohort studies of biologics-naïve RA, the typical finding has been a non-elevated risk of melanoma (supplementary table 1). To fully explore DMARDs as a confounder in this regard, we would need reliable information on csDMARD exposure ever since RA diagnosis and onwards until the diagnosis of melanoma or end of follow up. This information was not available in our data other than for a subset of individuals captured in the early RA register.

Instead, to address the issue of methotrexate as a potential confounder we performed additional analyses using data from the Prescribed Drug Register. 18,923 individuals among the TNFi-treated and the biologics-naïve started follow-up after July 2005 and 14,022 were ever treated with methotrexate 2005 though 2010. Using 4 levels (<1years through >3years) of methotrexate "exposure-years" during this time interval, and adjusting for TNFi treatment among other covariates, we found no indication that methotrexate was a confounder for invasive melanoma in our material. It is however important to realize that this analysis represents a quite narrow time-window of methotrexate exposure. The patients (in particular the TNFi-treated) may have a substantial and unknown history of DMARD exposure that in theory could impact the risk of melanoma.

In summary, although we cannot exclude the possibility that particular combinations or patterns of use of csDMARDs would increase melanoma risk, our data provide little evidence that this would be the case, or that the association observed with TNFi would primarily be driven by such confounding.

*Confounders extracted from medical files*

In study III, the matched study population (by age, year, *in situ* vs. invasive cancer at diagnosis, and county) was further characterized for breast cancer related prognostic factors by means of medical chart reviews. We hypothesized that TNFi-treated individuals would have less advanced cancer due to selection bias, but that these differences were too subtle to be captured through matching. Adjusting for these breast cancer characteristics (see section 4.1.5.4) in a model also adjusted for the comorbidities above indicated that they were modest confounders (HR changed from 0.8 (0.3-2.1) to 1.1 (0.4-2.8)), although the overall interpretation of the result did not change.

*Smoking*
We lacked information on smoking in all studies although we consider it an important confounder, in particular for NMSC (study II) and death (study IV). Instead we used a diagnosis of COPD in the patient register as a proxy for smoking in studies I-IV. COPD

was not a strong confounder in any of the multivariate models specified for the main analyses (changed the HRs less than 10%). However, it is likely that this variable is an imperfect proxy, and that smoking was inadequately adjusted for in our studies.

## 6.2 FINDINGS AND IMPLICATIONS

### 6.2.1 RA as a risk factor for skin cancer

#### *6.2.1.1 Melanoma*

We observed no increased risk of melanoma among biologics-naïve RA-patients compared to the general population (study I). There have been concerns of increased risk of melanoma in RA, due to factors linked to the immune-dysfunction *per se*, or immune-suppressive therapy [111, 171], similar to the increased risk of melanoma noted in organ transplant patients [166, 293]. Nevertheless, most observational studies previously investigating melanoma risk in biologics-naïve RA-patients, have found no increased risk compared with the general population. Our findings are in keeping with these prior studies. In summary, we observed that RA *per se*, or csDMARD treatment in RA, was not major risk factors for melanoma. This provides important "background" information for the interpretation of melanoma risk among RA-patients treated with TNFi (see section 6.2.2.1).

#### *6.2.1.2 Non-melanoma skin cancer.*

For biologics-naïve RA, we detected a doubled risk of SCC, and a 20% increased risk of BCC compared to the general population (study II). Profound immune-suppression is a well recognized risk factor for NMSC. For instance, organ transplantation has been associated with a 10-fold risk of BCC [162] and a 50-200-fold increased risk of SCC [133, 162, 172-173].

Prior investigations indicate a 20-100% increased risk of NMSC in biologics-naïve RA compared to the general population [108-109, 111-113, 177, 231, 242]. There are some differences between these studies and our study II that need to be pointed out. Most importantly, the reporting of NMSC was not mandatory in several of the study settings [111, 113, 176], leading to lower incidence rates and potentially differential reporting between RA (and other chronic diseases) and the general population. Also, most prior studies did not differentiate between different types of NMSC, so it has been unclear whether the increased risk of NMSC mainly pertains to the benign BCC or to more malignant types such as invasive SCC.

Our findings added to what was previously known about NMSC in RA, by differentiating between SCC and BCC. The finding of a doubled risk of SCC could either be attributed to immune system perturbation associated with the RA disease itself, or

to the (non-biologic) drug treatment, including methotrexate, sulfasalazine and anti-malarial DMARDs. In any case, this implicates that RA per se, is a more prominent risk factor for NMSC than TNFi treatment (see section 6.2.2.3).

### 6.2.2 TNFi as a risk factor for skin cancer

#### *6.2.2.1 Melanoma*

We found that TNFi treatment in RA was associated with a 50% increased risk of invasive malignant melanoma of the skin, but not of in situ melanoma or all-site cancer (study I).

Based on the fact that activation of the immune system is a key event in the tumor defense against melanomas [191], that immune-suppressive therapy is a known risk factor for development of melanoma in organ transplant patients [166, 293], and that isolated limb perfusion with TNF is a therapeutic approach used in advanced melanoma [188, 294-295], there have been concerns that TNFi treatment would increase the risk of melanoma in RA. This was partly supported by two previous studies from US/Canadian settings [113, 232] and one study from the Danish biologics register, although the latter had limited power [177].

We found an increased risk of invasive melanoma, but no increased risk of *in situ* melanoma. This finding could have alternative explanations, including low power of the *in situ* melanoma analysis. Detection bias could potentially contribute to the finding, but such detection bias is perhaps more likely to have overestimated *in situ* melanomas among TNFi treated patients owing to increased clinical vigilance. Finally, the biology of *in situ* and invasive melanoma may differ, which could explain our finding [296].

We detected a difference in relative risk (HR) of invasive melanoma among men and women. We carefully explored the risk among males to find factors which could explain this finding, such as sex-specific differences in socioeconomic status or residential area. None of these factors were strong confounders. We did not have information on factors related to the general "way of living" including diet, occupation and leisure, and sun/tanning habits. Such habits could possibly differ among men and women, and also interact with the risk of melanoma. None of the previous studies investigating melanoma among biologics-treated RA provide sex-specific rates [113, 230, 232]. Our finding is thus not corroborated by others and may be a chance finding.

#### *6.2.2.2 Melanoma risk in a clinical perspective*

In order to provide useful clinical information, any relative risk (or relative hazard) must be interpreted in the light of the underlying absolute risk. The observed 50% increase in relative risk, translates to 20 additional cases per 100 000 person years. In

other words, if the observed association with TNF inhibitors reflects causality, thousands of rheumatoid arthritis patients must be treated for one year for one melanoma to be attributable to the TNF inhibitor treatment.

We investigated all-site cancer mainly to put melanoma risk in perspective. Melanomas comprised 7% of all incident cancers in our study. When excluding melanomas from the all-site analyses, the HRs for all-site cancer were identical. This implies that our finding of an increased risk of melanoma associated with TNFi treatment does not alter the overall risk-benefit balance of TNFi treatment in most patients, but perhaps do so in a subset of high-risk patients.

Against the above, the beneficial effects of TNFi treatment will in most cases outweigh the small increase in risk of melanoma. Our finding may however, shift the risk benefit balance in patients at high risk, such as those with a history of melanoma. Given the excellent prognosis of melanomas if detected early, increased clinical vigilance is probably advisable in such patients if treatment with TNF inhibitors is considered. The increased risk of melanoma in our population of RA-patients with, for the most part, fair skin type, may not be generalized to other settings with different skin types and/or different tanning habits. This is supported by a pooled analysis of TNFi-associated melanoma risk across different European biologics-registers (*Unpublished data, Joachim Listing*).

#### *6.2.2.3 Non-melanoma skin cancer*

For TNFi-treated RA, we found a 20% increase in risk of *in situ* SCC among RA patients treated with TNFi, but no increased risk of invasive SCC, or of BCC, compared to biologics-naïve RA (study II). The increased risk of SCC but not BCC in our study have plausible biologic explanations since SCC and BCC display partly different genetic hallmarks and somewhat different risk factors as outlined in section 3.7.2.

TNFi has been suggested as a risk factor for NMSC, supported by case reports [297-298] and some observational [111, 113, 233, 241, 280], as well as clinical trial data [229].With respect to observational studies, our findings are partly compatible with two studies using the US National Data Bank for Rheumatic Diseases (NDB) and one recent US study using administrative data, although SCC and BCC were not studied separately and the incidence rate of NMSC combined were substantially lower than in our study [111, 113]. Studies in European settings have not confirmed an increased risk of NMSC associated with TNFi-treatment in RA [177, 242], which may have several explanation including low power and the inability to study SCC and BCC separately (see section 3.8.3.3).

Basal cell cancer is reported to the national cancer register nationwide only since 2004, which limits any inference of TNFi-exposure and BCC to those starting TNFi – treatment from 2004 and onwards.

Non-melanoma risk in a clinical perspective

The 20% increased risk of SCC associated with TNFi was mainly attributable to *in situ* lesions. This may indicate that clinicians and patients are extra observant of skin lesions in the context of TNFi treatment, i.e. the finding may be explained partly by detection bias. On the other hand, the fact that we found no signs of increased risk of BCC (which could be expected to be at least as sensitive to detection bias as SCC) , speaks in favor of a true increased risk of SCC.

SCC risk largely depends on age, and monitoring for this potential adverse event may have a higher pay-off in certain age-groups. Translating our finding of increased risk of SCC into an absolute risk, a thousand patients in the age group 60+ need to be treated with TNFi during a year in order for one SCC to emerge as an adverse event. In the age group 80+ the corresponding number is approximately 200. Nevertheless, any increased risk of SCC in the context of TNFi treatment would be smaller than the risk associated with the risk associated with RA per se.

### 6.2.3 Recurrent breast cancer and TNFi treatment

With a follow up of 5 years, we found no difference in the risk of breast cancer recurrence between TNFi-treated and matched biologics-naïve patients with RA and a history of breast cancer at a mean 9.5 years prior to inclusion. The all-cause mortality was similar for the two groups (study III).

Ever since their introduction, there have been concerns that TNFi might impact the risk of cancer development, or alter the risk of recurrence of previous cancers. Based on these concerns and due to limited clinical evidence, most treatment guidelines advocate restrictive use of TNFi in patients with a history of cancer during the last five or ten years [243-244].

The two studies previously published had focused on recurrent cancer from of all types, and lacked baseline data on cancer prognostic factors, i.e. channelling bias could not be characterized [240, 245].

In patients with a history of cancer, the decision to initiate or abstain from TNFi is based upon a clinical judgment of the risk/benefit balance. A patient with a history of a recent, larger or high grade tumor may be less likely to receive TNFi compared to an individual with a breast cancer of better prognosis. In order to accurately study the difference in recurrent cancers among TNFi-treated and biologics-naïve, great caution must be taken to eliminate differences in patient and cancer characteristics at diagnosis

of the index tumor. Since breast cancer is the single most important malignancy type in middle-aged women, we focused on this particular malignancy rather than all-site cancer. We used a matched cohort design, which allowed us to condition upon one of the most important risk determinants (time since breast cancer), as well as year of diagnosis, age at diagnosis county and *in situ* versus invasive cancer. We also reviewed medical files in all individuals, characterizing the index cancers at diagnosis and RA disease at baseline and during follow-up.

The chart reviews indicated that there was some channelling of patients at a high risk of recurrent cancer away from TNFi treatment. The index cancers among the biologics-naïve were slightly more likely to have nodal engagement and were more often treated with mastectomy. Chemotherapy was more common among the biologics-naïve compared to the TNFi-treated. Chart review also indicated that around 10% of the biologics-naïve patients did not start TNFi due to perceived high risk of breast cancer. Although these differences were small, and based on information for some variables with substantial and differential missing, they indicate that the TNFi-treated had a slightly less advanced index cancer on a group level. Adjusting for these differences had, however, little impact on our HRs.

Stratifying on time since index breast cancer until TNFi -treatment start did not reveal any significant difference in HRs between the two time intervals, although precision was low (only 15% of our study population started TNFi treatment within 5 years of their breast cancer).

We hypothesized that TNFi may increase the risk of recurrent breast cancer. However, there are experimental data which support also the opposite hypothesis that, TNFi treatment could be protective against tumor progression and spread (see section 3.7.3.2). It is likely that blocking of the physiologic effects of TNF has the potential to either promote or protect against cancer progression, depending on factors such as the genetic and/or pathologic subtype of the tumor, and other patient–related factors such as drug treatment, age, weight or diet [154]. This “unpredictable” impact is reflected also in clinical data. Low levels of TNF have been associated with less tumor progression in patients with locally advanced breast cancer [299], and at least one clinical trial has evaluated the safety of TNFi as treatment of breast cancer (although with inconclusive efficacy) [194]. On the other hand, clinical data have linked high levels of TNF with disease-free survival in patients with metastatic breast cancer [300], indicating that TNFi treatment might be detrimental in advanced breast cancer. In summary, the finding of no increased risk of recurrent breast cancer is compatible with the multifaceted impact of TNFi on cancer initiation and/or cancer recurrence.

Although our study included all eligible RA-patients with TNFi treatment with a history of breast cancer in Sweden during the study period, the study was still limited due to

low power. With α=0.05, a study of our design would require approximately 120 patients in each treatment group (i.e., our sample size) to have 80% power to detect a doubled risk, but 350 patients in each treatment group to detect a 50% increased risk.

#### *6.2.3.1 The risk of recurrent breast cancer in a clinical perspective*

Within the group of breast cancers which were included in our study, no increased risk was detected. This does not preclude the possibility of increased risk among a certain subset of breast cancers, either defined by genotype or molecular subtype. Further, to be included in our analyses the breast cancer had to be in remission at start of follow-up. Our study population was thus inherently restricted to women surviving their breast cancer up until the time point of start of TNFi treatment. For other cancer types, such as cancers of the lung, pancreas, and brain, radical treatment is often not achieved and 5-year survival is typically lower than for breast cancer [301]. It cannot be excluded that TNFi treatment could be more detrimental in terms of recurrence in cancer types with less favorable prognosis. Our study had limited power. However, we may conclude that, given a true increased risk of recurrent breast cancer associated with TNFi, this risk is less than doubled.

### 6.2.4 Cancer stage at presentation and post cancer survival

In study IV we observed that cancers occurring in RA-patients who are, or have been, treated with TNFi do not present with any marked difference in stage at presentation compared to cancers occurring in RA patients never treated with biological drugs. Overall post-cancer survival was similar between TNFi-treated vs. biologics-naïve, although some of the site-specific HRs was (non-significantly) below unity.

Prior to our study, there were only limited data on mortality rates among patients treated with TNFi and the outcome measure used (death) does not allow for any discrimination between cancer incidence and cancer survival ("case fatality") [302].

The study of post-cancer survival has caveats. In the context of the more frequent health-care visits and increased vigilance, cancers among TNFi-treated may be detected at an earlier stage (see section 6.1.2.6). To be able to make valid inference about survival in our study, we therefore needed to present the distribution of stage at presentation. To this end, we used information on stage at diagnosis collected from two separate sources: the medical charts and TNM stage available in the national cancer register. With the possible exception of distant metastases (stage IV), both the matched register-derived data and the data retrieved from the medical charts revealed similar stages at presentation among the TNFi-treated and the biologics-naïve for all cancer sites combined. Nevertheless, some site-specific differences in stage distribution were detected, with a tendency toward a less advanced stage at presentation among the TNFi-treated cancers (apart from malignant melanomas). Together with the fact that it

was not possible to fully adjust for stage in the assessment of cancer survival, this might explain the tendency for some of the HRs of post-cancer survival to be slightly below 1.

Considering the difficulty of assigning and comparing causes of death among individuals with multiple chronic diseases and the possibility of competing causes of death, we used all-cause mortality as the main outcome measure (though analysis restricted to cancer-specific deaths resulted in similar HRs [data not shown].

#### *6.2.4.1 Post-cancer survival in a clinical perspective*

The mean follow-up starting from cancer diagnosis in our study was 4 years among TNFi-treated (maximum 10 years), which is not fully sufficient to detect long-term effects. On the other hand, the increased force of mortality from cancer is most pronounced during the first years following cancer diagnosis [303]. In this perspective our findings may be reassuring to patients and physicians concerned about the impacts of prior TNFi treatment on a current cancer. It should be noted however, that our findings do not provide evidence regarding the effects of continuing treatment with TNFi following a diagnosis of cancer (in our study, most patients discontinued TNFi at cancer diagnosis). Such a study would require a careful investigation of cancer-related prognostic factors on a case-by-case level similar to the method in study III.

# 7 CONCLUSIONS

In this thesis I have investigated skin cancer incidence, breast cancer recurrence, and post-cancer survival in RA patients treated, and never treated, with TNFi. My overall conclusion is that TNFi treatment has a generally favorable risk-benefit profile among individuals selected for treatment in clinical practice by Swedish rheumatologists.

We found a 50% increased risk of invasive melanoma, and a 20% increased risk of SCC associated with TNFi treatment. At first glance, these findings may seem dramatic. But it should be kept in mind that, taking melanoma as an example, the risk increase translates into approximately 20 extra cases over 100,000 person-years of treatment. This makes melanoma a very rare side effect of TNFi treatment, and as such, it must be viewed in light of the excellent effectiveness of these drugs in RA. We observed that patients treated with TNFi displayed similar post-cancer survival compared to RA-patients never treated with biological drugs. Finally, TNFi treatment appears not to increase the risk of breast cancer recurrence, although larger studies will be needed to confirm this with certainty. None of the four studies indicated particularly strong confounders or substantial channelling bias, although to some extent, bias due to both may still remain due to the use of observational study designs.

Needless to say, the conclusion of a favorable risk-benefit balance relates only to the specific aspects that we have investigated in the four studies. It is not unlikely that other aspects of TNFi-related safety, maybe with the selection of particularly vulnerable populations or populations receiving higher doses of TNFi, would tilt that balance.

Specific conclusions of the four studies:

- RA patients never treated with biological drugs suffer no increased risk of melanoma, compared to the general population (study I).
- RA patients never treated with biological drugs suffer a doubled risk of SCC and a moderately increased risk of BCC compared to the general population (study II). This implicates that RA *per se*, or the use of csDMARDs in RA, are more prominent risk factors for NMSC, than TNFi treatment.
- TNFi treatment increases the risk of invasive melanomas by 50%, but not of *in situ* melanomas or of invasive cancers overall (study I).
- TNFi treatment increases the risk of SCC with 20% but has no impact on risk of BCC (study II).
- The increased risk of SCC associated with TNFi treatment was confined to *in situ* (as opposed to invasive) lesions, which indicates the possibility of surveillance bias (study II).

- TNFi treatment that was initiated on average a decade after breast cancer diagnosis did not increase the risk of breast cancer recurrence, compared to matched RA patients never treated with biological drugs (study III).
- Cancers occurring in RA-patients who are, or have been, treated with TNFi do not present with any marked difference in stage at presentation or post-cancer survival, compared to cancers occurring in RA patients never treated with biological drugs (study IV).

# 8 FUTURE PERSPECTIVES

Many scientific questions may be explored in the context of RA, RA drug treatment, and cancer (if not hindered by the imminent threat from the new EU General Data Protection Regulation [304]). The indications of increased risk of melanoma and NMSC and the non-increased risk of recurrent breast cancer need to be confirmed in larger cohorts, and in cohorts from other populations reflecting other background risks (RA disease activity, comorbidities and DMARDs) and other TNFi regimes. *Recurrent cancer* (all types) and the unremarkable *post-cancer survival* in TNFi-treated RA could also be explored under the hypothesis that survival is increased, i.e. that TNFi exerts an anti-cancer effect.

More specific questions regarding all outcomes suggested above should also be posed:

- What role do ACPA- and/or RF-status play in the risk of cancer-related outcomes in TNFi-treated RA? What is the impact of smoking as a confounder or effect modifier?
- Are there certain gene patterns which make some RA individuals prone to, or less susceptible to, certain cancer related outcomes in the context of TNFi treatment?
- Are the risks differential between the specific TNFi drugs?
- Could measuring of serum levels of TNFi or other csDMARDs (therapeutic drug monitoring) be of value to pinpoint patients with increased risk of melanoma or NMSC?

Some of the above listed research questions are simply awaiting the accrual of more person-years of follow-up in our registers, or on the international collaboration between registers to gain power. Such collaborative efforts have already been initiated in the European setting [305] and studies are underway (e.g. of melanoma and lymphoma). The pooling of data across registers is not without challenges. There are known differences in the RA-patients, both treated and untreated with TNFi, with respect to smoking, comorbidities and disease activity at treatment start [306-307]. The methods of data collection and variable definitions also vary across registers. Nevertheless, the augmented power provided by the pooling of data could provide a

possibility to study subgroups of patients (e.g. different cancer types) or important determinants of adverse events such as cancer.

The linkages of SRQ-data to national quality of care registers for cancer or other diseases provide new possibilities of detailed clinical outcome data which reduce the need for case-by case chart reviews. Further, biological material will be needed for some of the future research mentioned above.

Finally, the emergence of new therapeutic options in RA presents us with a whole new arena to explore. There will be a need for the same structured studies of these drugs in the context of RA and cancer. This applies to both the biosimilars introduced as a more affordable generic treatment compared to the approved TNFi, and the novel therapeutic strategies offered by the JAK-inhibitors. In order to assess these new drugs, csDMARDs and TNFi will by necessity act as comparators. It will thus be important to continue the safety (and efficacy) evaluations of the TNFi in parallel to the introduction of newer substances. The same applies for the csDMARDs. Many safety aspects of the anchor drugs such as glucocorticoids, methotrexate, sulphasalazine and anti-malarial drugs are still insufficiently elucidated. Hybrids between the classical randomized clinical trial and observational study design, such as "register-enriched clinical trials" [308] "effectiveness clinical trials" [52], "pragmatic clinical trials" [309-310] provide attractive alternatives which may open up a new horizon for clinical pharmacology and clinical epidemiology.

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# 10 SUPPLEMENTARY MATERIAL

**Supplementary table 1**. Seminal studies of melanoma risk in rheumatoid arthritis (RA)-patients conventional synthetic or biologic disease modifying anti-rheumatic drugs (DMARDs)

| Study & Year of publ. | Setting & design | Study period | RA population | Drug treatment | Mela-noma | Relative risk of melanoma in patients with RA |
|---|---|---|---|---|---|---|
| Gridley 1993 | Sweden Population-based | 1965-1984 | n=11,683 | Not specified (pre-biologic era) | 12 | **SIR:** 0.9 (0.5-1.6) |
| Mellemkjaer 1996 | Denmark Population-based | 1977-1991 | n=20,699* | Not specified (pre-biologic era) | 37 | **SIR**: 1.1 (0.8-1.5) |
| Thomas 2000 | Scotland Population-based | 1981-1996 | n=7,080 | Not specified (pre-biologic era) | 2 (m) 26 (f) | **SIR**: 0.3 (0.0-1.2) male; 1.2 (0.8-1.8) female |
| Askling 2005 | Sweden Population-based | 1999-2003 | a) n=3,703 and 5,3067 b) n=4,160 (TNFi) | a) Biologics-naive b) TNFi-treated | a)124 b)1 | a) **SIR:** RA 1.2 (1.0-1.4) and 0.9 (0.2-2.2) b) **HR**: TNFi treated versus biologics naive: 0.3 (0.0-1.8) |
| Setoguchi 2006 | US & Canada Community-based | 1994-2004 | Pooled RA cohort: n=8,458, 65 yrs + | Biologics-treated**: 14%; MTX: 86% | 29 | **SIR** RA, including biologics-treated: 2.3 (1.6-3.2) |
| Wolfe & Michaud 2007 | US Community-based | 1998-2005 | n=13,001; | Biologics-treated**: 41%; MTX: 57% | 22 | a) **SIR** RA, including biologics-treat.: 1.7 (1.3-2.3) b) **RR** TNFi and versus biologics-naive RA: 2.3 (0.9-5.4). |
| Abásolo 2008 | Spain, Community-based | 1999-2005 | n=789 | csDMARDs | 1 | **SIR**: 3.8 (0.1-21.0) |
| Buchbinder 2008 | Australia Community-based | 1986- 1995 | n= 458 | csDMARDs, (100% MTX) | 7 | **SIR:** 3.0 ( 1.2-6.2) |
| Hellgren 2010 | Sweden Population-based case-control | 1997-2006 | n=6,745 | Unselected incident RA | 11 | **RR** 1.0 (0.5-2.0) |
| Perkins 2012 | Review and meta-analysis | 1990-2010 | 1,351,061 person-yrs | csDMARDs | 601 | **SIR** 1.0 (0.9-1.1) |

*SIR= Standardized Incidence Ratio vs. the general population cancer incidence*

*RR= Relative Risk*

*HR= Hazard Ratio*

*csDMARD=conventional synthetic Disease-Modifying Antirheumatic Drug, TNFi=Tumor Necrosis Factor inhibitor, MTX= Methotrexate, GenPop=General Population, Inc=Incident, Prev=Prevalent, m=male, f=female*

**Cohort includes patients with rheumatoid arthritis, juvenile rheumatoid arthritis and unspecified rheumatoid arthritis.*

*** Anakinra or (predominantly) TNFi-treated*

**Supplementary table 2 page 1. Extraction form for clinical variables study III**

## Basdata och RA-sjukdomen

**Kontroll Ja** ☐ **Nej** ☐ **Personnummer**

**RA site**

***Matchningsdatum=första TNFi***

RA-duration (fram till matchningsdatum) ☐ <3år ☐ ≥3år ≤ 10år ☐ >10år ☐ Uppgift saknas

Seropositivitet ☐ ja ☐ nej ☐ Uppgift saknas

Erosiv sjukdom ☐ ja ☐ nej ☐ Uppgift saknas

**DMARDS** Antal under åren (fram till diagnos av indexcancer eller BIO?) ☐ 0 ☐ 1-2 ☐ 3+ ☐ Uppgift saknas

SSZ ☐ AU ☐ AZT ☐ HXK ☐ MTX ☐ Cik ☐ Cyk ☐ LFD ☐

Annat

**Sjukdomsaktivitet** året före matchningsdatum ☐ låg ☐ måttlig ☐ hög ☐ framgår ej

**Regelbunden COX-hämmare (4 veckor)** året före m-datum ☐ ja ☐ nej ☐ Uppgift saknas

**P.o kortison** under året före matchningsdatum
(minst 4 sammanhängande veckor) ☐ ja ☐ nej ☐ Uppgift saknas

**DMARDS** under året före matchningsdatum ☐ ja

☐ nej ☐ Uppgift saknas

**Supplementary table 2 page 2. Extraction form for clinical variables study III**

## Indexcancer

**Personnummer**

**Diagnosdatum**

**Diagnos enligt journal**

**Pure mucinous, pure tubular, pure medullary or pure papillary** ☐ ja, ☐ nej ☐ Uppgift saknas

☐ **Höger** ☐ **Vänster** ☐ **Multifokalt** ☐ Uppgift saknas

**Tumörstorl**** ☐ ≤1cm ☐ 1-2cm ☐ 2,1-3cm ☐ 3,1-5cm ☐ >5cm ☐ Uppgift saknas

**Körtlar** ☐ 0 ☐ 1-3 ☐ 4-9 ☐ >9 ☐ Odef. antal ☐ Uppgift saknas

**Spridd sjukdom vid diagnos*** ☐ja ☐nej ☐ Uppgift saknas

*Fjärrmetastaser inklusive supraclaviuculära eller kontralaterala körtlar eller kontralateralt bröst

**Tumörstorlek gäller största tumören vid multifokala förändringar

**TNM** ☐Uppgift saknas

**Tumor Grade** (”lågt diff”= grade3) ☐ 1 ☐ 2 ☐ 3 ☐ Uppgift saknas

*Histologisk grad (tubulär formation, grad av kärnatypi, mitosaktivitet) Bloom-Richardson (B-R) or Scarff-Bloom-Richardson grade 1-3).*

**HER2-receptor positiv** ☐ja ☐nej ☐ Uppgift saknas

**Postmenopausal** ☐ja ☐nej ☐ Uppgift saknas

**Östrogenreceptorstatus** ☐ pos ☐ neg ☐ Uppgift saknas

**Progesteronreceptorstatus** ☐ pos ☐ neg ☐ Uppgift saknas

## Behandling av indexcancer

Kirurgi , bröstbevarande ☐

Kirurgi Mastektomi ☐ ☐ Uppgift saknas

**Hormonell** behandling (tamoxifen eller aromatashämmare) ☐ ja, ☐ nej ☐ Uppgift saknas

**Herceptin** ☐ ja, ☐ nej ☐ Uppgift saknas

**Antal år med hormonell behandling**_____

**Supplementary table 2 page 3. Extraction form for clinical variables study III**

**Adjuvant cytostatika**

☐ nej orsak

Adjuvant cytostatika **1:a generationen- CMF** ☐

Adjuvant cytostatika **2:a generationen- CAF, FEC, annan antracyklinbaserad** ☐

Adjuvan cytostatika **3:e generationen, ovanstående med tillägg av taxaner** ☐

Adjuvant cytostatika, oklart vilken typ ☐ ☐ Uppgift saknas

**Adjuvant strålning** ☐ ja, ☐ nej ☐ Uppgift saknas

## Bedömning inför TNFi start eller vid motsvarande datum för kontrollerna

*Patient som erhåller TNFi:*

I cancerremission enligt journal (onkolog eller RA-journal) ☐ ja ☐ nej ☐ Uppgift saknas

**Om nej eller uppgift saknas, orsak till att TNFi initieras ändå:**

☐ Svår RA som behöver behandlas trots risken, i samförstånd mellan patient och läkare

☐ Svår RA och risken för progress/återfall bedöms som låg

☐ Svår RA, compassionate use. Patienten är svårt sjuk och ett återfall eller progress av bröstcancer är av underordnad betydelse

☐ Resonemang saknas (inbegriper att det heller inte går att utläsa underförstått i journaltexten)

**Om ja, orsak till att TNFi initieras:**

☐ Svår RA som behöver behandlas trots risken, i samförstånd mellan patient och läkare

☐ Svår RA och patienten uttrycker stark önskan att erhålla behandlingen trots avrådan från läkare

☐ Svår RA och risken för progress/återfall bedöms som låg

☐ Resonemang saknas (inbegriper att det heller inte går att utläsa underförstått i journaltexten

**Supplementary table 2 page 4. Extraction form for clinical variables study III**

## Bedömning inför TNFi start eller vid motsvarande datum för kontrollerna (forts.)

*Kontrollpatient (Har ej erhållit TNFi)*

I cancerremission enligt journal (onkolog eller RA-journal) ☐ ja ☐ nej ☐ Uppgift saknas

Om ja, varför erhåller patienten inte TNFi (tom matchningsdatum)?

☐ Svår RA som skulle behöva TNFi, men man väljer att avstå pga oklar risk för återfall. I samförstånd mellan patient och läkare.

☐ Svår RA men patienten uttrycker stark önskan att avstå TNFi pga risk för återfall

☐ Indikation saknas (för låg sjukdomsaktivitet)

☐ Kontraindikation eller relativ kontraindikation annan än maligniteten, vilken:

☐ Resonemang saknas (inbegriper att det heller inte går att utläsa underförstått i journaltexten)

## Läkemedelsbehandling från matchningsdatum till uppföljningens slut

**Regelbunden COX-hämmare (4 veckor)** ☐ ja ☐ nej ☐ Uppgift saknas

**P.o kortison** (minst 4 sammanhängande veckor*) ☐ ja ☐ nej ☐ Uppgift saknas

**DMARDS** ☐ ja

☐ nej ☐ Uppgift saknas

***Minst 3 månader efter initiering av TNFi eller motsv. datum för kontrollerna**

**Supplementary table 2 page 5. Extraction form for clinical variables study III**

## Relaps under uppföljningstiden (från matchningsdatum tom sista anteckning)

**Diagnosdatum**

**Diagnos enligt journal**

**Symptom som orsak till diagnos** Ja ☐ Nej ☐

**Upptäckt en passant vid besök hos reumatolog** Ja ☐ Nej ☐

**Upptäckt på grund av kontroller relaterat till TNFi** Ja ☐ Nej ☐

**Övriga orsaker** _____ ☐

**Uppgift saknas** ☐

**Stadium vid diagnos av återfallet (ringa in)**

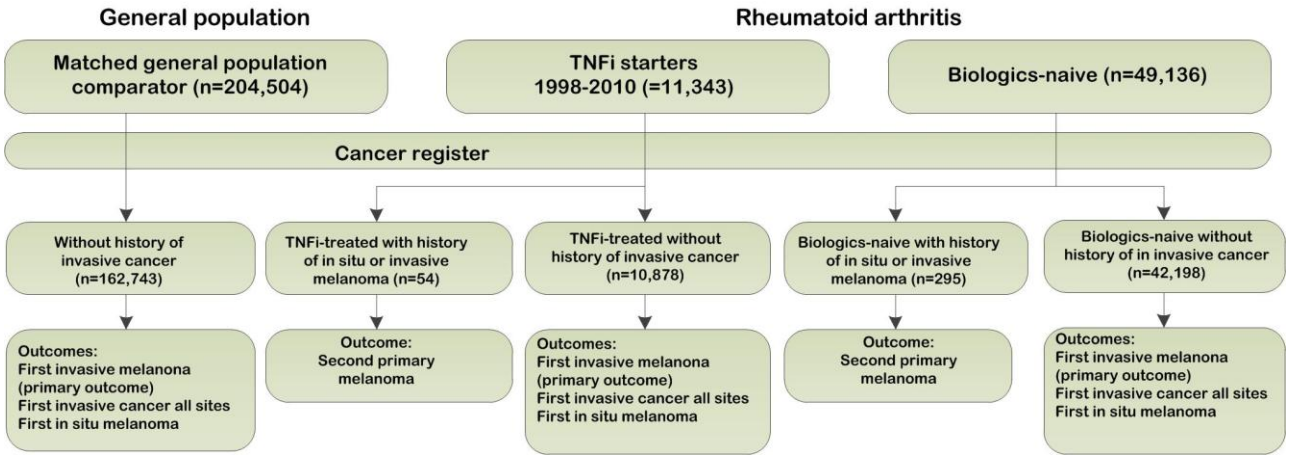
1 ärrvävnad
2 ipsilateralt bröst
3 ipsilateral axill
4 ipsilateralt supraclav
5 kontralateral axill och supraclav
6 kontralateralt bröst
7. Fjärrmetastaser eller lokoregionalt avancerad
8. Uppgifter om stadium saknas

**Ej tecken till återfall under uppföljningstiden från matchningsdatum** ☐

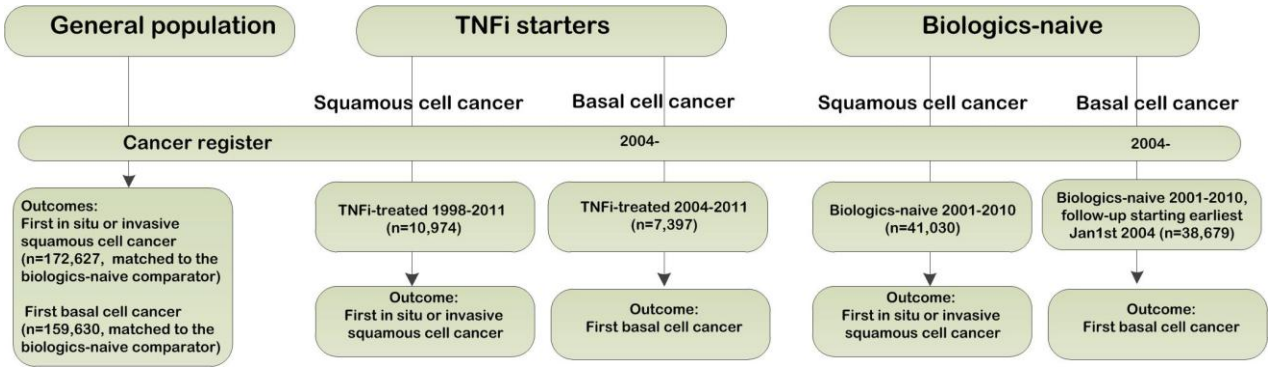
**Supplementary table 3.** Characteristics of the index breast cancer (occurring prior to start of follow-up), extracted from medical files in 120 TNFi-treated and 120 biologics-naïve patients with rheumatoid arthritis and a history of breast cancer.

| | Biologics-naïve n=120 | TNFi-treated n=120 |
|---|---|---|
| **Age at cancer diagnosis (IQR)** | 55 (14) | 54 (13) |
| **Year of cancer diagnosis (%)** | | |
| 1960-1980 | 9/120 (8) | 9/120 (8) |
| 1981-1990 | 23/120 (19) | 26/120 (22) |
| 1991-2000 | 71/120 (59) | 68/120 (57) |
| 2001-2007 | 17/120 (14) | 17/120 (14) |
| **Cancer stage (%)** | | |
| In situ | 24/120 (20) | 24/119 (20) |
| Invasive | 96/120 (80) | 96/119 (80) |
| **Size, invasive tumors (%)** | | |
| ≤2 | 62/96 (66) | 65/96 (68) |
| 2,1-5 cm | 11/96 (11) | 12/96 (13) |
| >5 cm | 2/96 (2) | 2 /96 (2) |
| Undefined | 21/96 (22) | 17/96 (18) |
| **Histologic type (%)** | | |
| Ductal carcinoma | 52/ 67 (78) | 57/78 (73) |
| Other | 15/67 (22) | 21/78 (27) |
| **Histologic grade*** | | |
| 1 | 18/66 (27) | 24/68 (35) |
| 2 | 31/66 (47) | 24/68 (35) |
| 3 | 17/66 (26) | 20/68 (29) |
| **Positive lymph nodes (%)** | 23 /83 (28) | 13/89 (15) |
| **Estrogen receptor positive** | 52/67 (78) | 48/62 (77) |
| **Surgical treatment** | | |
| Breast conserving surgery | 44/100 (44) | 60/105 (57) |
| Mastectomy | 56/100 (56) | 45/105 (43) |
| **Radiation therapy** | 53/104 (51) | 54/107 (50) |
| **Anti-estrogen therapy** | 44/102 (43) | 37/104 (36) |
| **Chemotherapy** | 18 /97 (19) | 12 /106 (11) |
| **Any recurrence (in remission) before start of follow-up** | 9 | 4 |
| **Predicted 10-year risk of breast cancer relapse, median (IQR) **** | 19 (14) | 18 (10) |

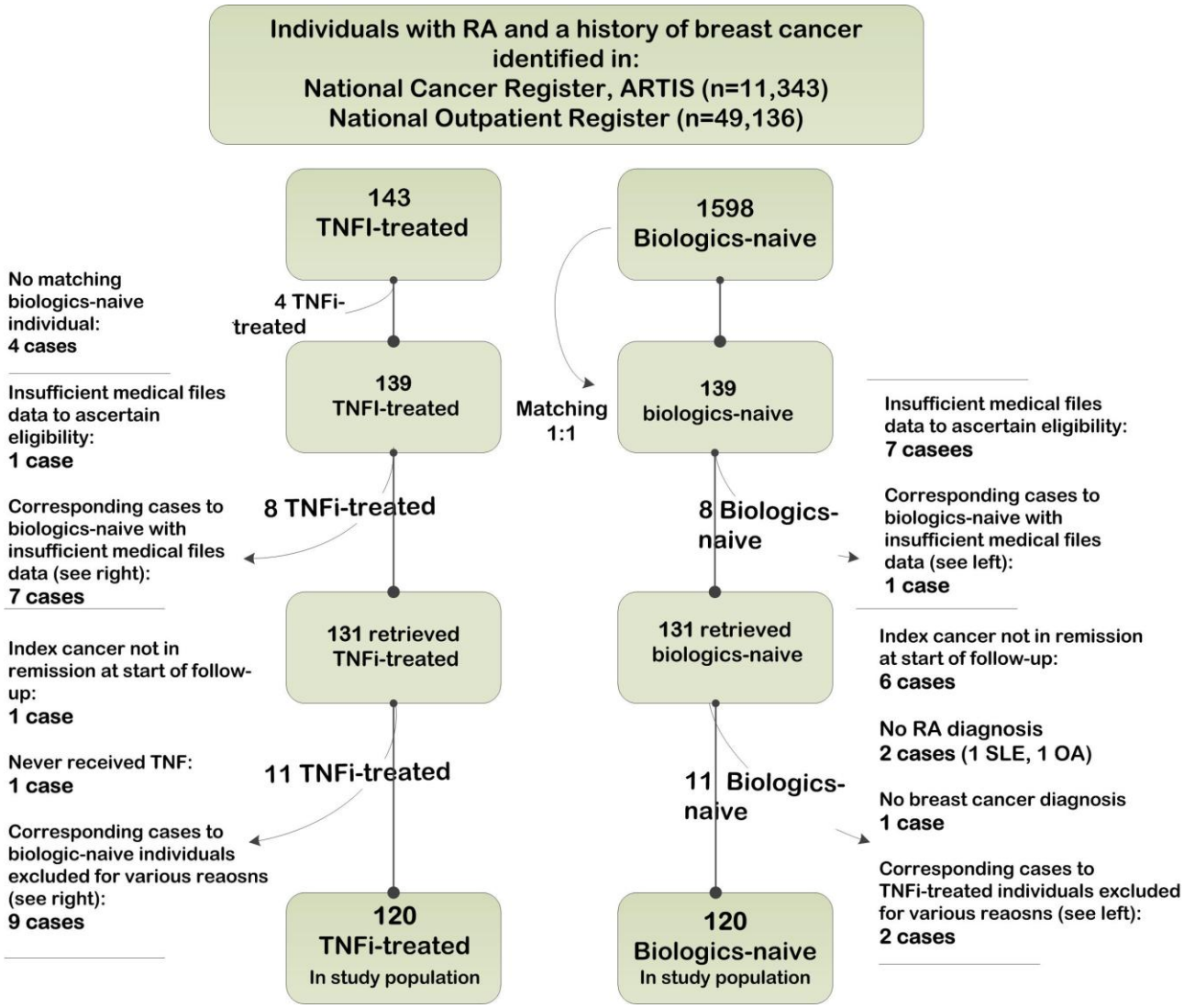
*Table shows numbers (percent) unless otherwise stated. Information of several variables was missing or insufficient for validation in the medical files. Individuals with missing information were subtracted, resulting in different denominators across rows.*
**Highest category (grade 3)=poorly differentiated disease*
***Calculated at diagnosis of index-cancer among 74 TNFi-exposed and 69 biologics-naïve invasive tumors, using Adjuvant! Online risk score. The model uses information on age, general health status (based on comorbidities listed in Appendix 2), estrogen-receptor status, tumor grade, tumor size, number of malignancy positive lymph nodes and use of hormonal therapy, chemotherapy, or both, to calculate each individual's predicted 10-year risk of breast cancer recurrence (http://www.adjuvantonline.com/index.jsp).*



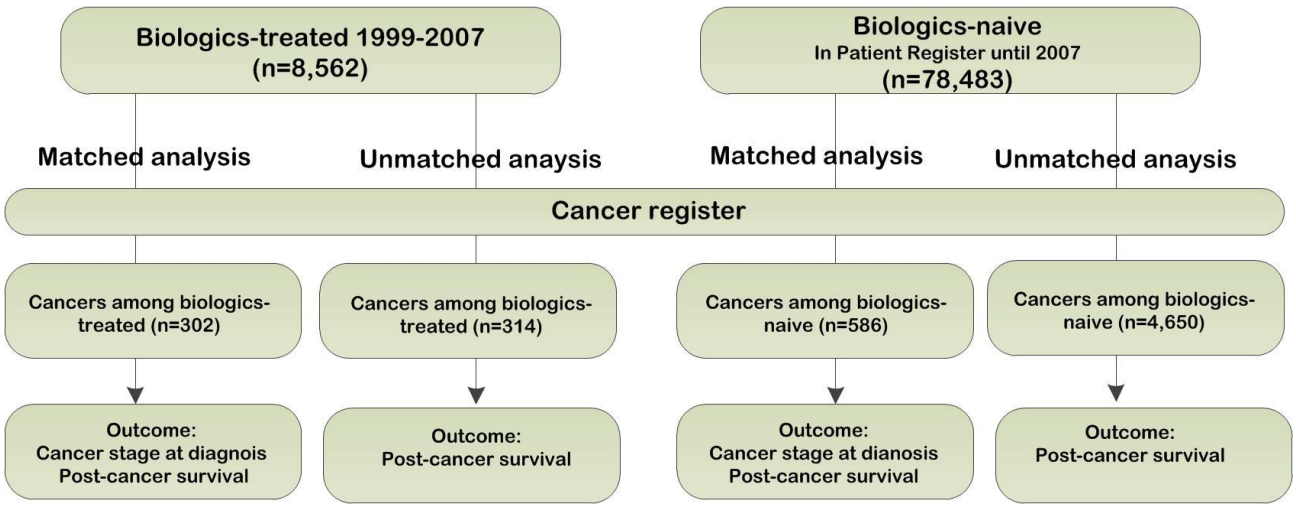

**Supplementary figure 1. Flowchart of study population in study I**




**Supplementary figure 2. Flowchart of study population in study II**




Supplementary figure 3. Flowchart of study population in study III




Supplementary figure 4. Flowchart of study population in study IV