JANUS serumbank

Special issue

The Janus Serum Bank - From sample collection to cancer research

Guest Editor: Joakim Dillner
Writing group: Hilde Langseth, Randi Gislefoss, Jan Ivar Martinsen, Adele Stornes, Marianne Lauritzen, Aage Andersen and Egil Jellum
Analysis: Jan Ivar Martinsen
Layout and design: Børge Sildnes
Correspondence to: Hilde Langseth (hilde.langseth@kreftregisteret.no)

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Acknowledgements

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Summary

The Janus Serum Bank is one of the world's oldest and largest population-based research biobanks. It is available for researchers with specific interest in cancer research. Janus has been involved in a large number of projects in cancer research that have had implications on early detection of cancer and treatment regimes for cancer patients. Janus was established in 1973 to collect and store blood samples from a large number of presumably healthy individuals for future use in cancer research. The intention was to look retrospectively to the preclinical stage in cancer patients, or look further back at the latent period before the tumor itself has developed, to detect important factors in the pathogeneses or aetiology of cancer.

At present the Janus repository consists of 674 386 vials from 452 376 blood draws among 316 951 donors. The cohort members consist of I) persons undergoing routine health examinations in different counties in Norway (more than 90%), and II) Red Cross blood donors from Oslo and the surroundings (less than 10%). For a small group of previous Janus donors referred to the Norwegian Radium Hospital for cancer treatment, samples are collected before treatment starts.

The Janus cohort is annually linked by personal identification numbers to the Cancer Registry of Norway to identify new cancer cases. As of 31 December 2008, the number of incident cancer cases in Janus was 48 076. Of these 26 216 cases were identified among male donors and 21 860 among female donors, 3396 of which have donated post-diagnostic samples at the Norwegian Radium Hospital. The most common cancer site among female donors is breast cancer (n=6407) and among males, prostate cancer (n=6521).

The long follow-up, the large number of cancer cases and the close interface to the Cancer Registry of Norway make the Janus biobank a unique sample collection for cancer research.

1 Development of the Janus Serum Bank

Historical background and establishment

Olav Torgersen

The JANUS project was established in 1973 on the initiative of the Norwegian professor in pathology, Olav Torgersen (1907–1978). Back in 1975 he wrote about the intention of the Janus project (1) and referred to the longstanding maxim that "prevention is better than cure". From the onset, Janus has been dedicated to cancer research and the investigation of cancer aetiology and early detection, by measuring biochemical and immunological changes several years before the patient's diagnosis. The intention was to collect and store blood samples from a large number of presumably healthy individuals for future use in cancer research. The Janus project was named after the Roman god, Janus. The dual-faced image symbolises the multipurpose nature of the project for retrospective as well as prospective investigations of cancer. Most human cancer studies involve data from patients after the tumor has been diagnosed. The opportunity to detect important factors in the pathogeneses or aetiology of cancer may then be lost. Janus enables retrospect assessment of the preclinical stage in cancer patients, or the latent period before the tumor has been established (1). The idea was first raised in 1957 by a group of Norwegian researchers, but they did not get any support or funds to establish a biobank. A further aim was to collect post-diagnostic serum samples from different hospitals where the cancer patients were treated, to investigate biochemical changes after diagnosis. This collection was feasible only at the Norwegian Radium Hospital (NRH). Due to financial issues, there was a break in the sample collection from 1992 to 1997.

The Janus initiative was a collaboration between former National X-ray examinations, the National Health Screening Services, Ullevål University Hospital and the Cancer Registry of Norway (CRN). The material in Janus consisted of the biological samples and the data attached to them. The health examinations were administrated by the National Health Screening Services (2;3), who sent the lists of participants to the CRN. They were responsible for the data handling, and prepared lists of the Janus donors and allocated placing of the samples in storage. The sample collection was administered by different health institutions and analysed at Ullevål University Hospital for risk factors for cardiovascular diseases. Residue material was sent to the Janus Serum Bank.

Vil kreft kunne bli påvist ved blodprøve?

Enestående prosjekt går ut på å finne krefttegn i blodet

Dosent Egil Jellum og Anne-Karine Thorsrud

Meticulous collection of samples over the last decades from a large number of volunteers at several health institutions, and the great willingness of volunteers to donate blood for the purpose of cancer research, have made Janus one of the world's oldest and largest biobanks. From the outset, the philosophy of Janus was that the data and samples from the Janus biobank were invaluable and should be made available to researchers worldwide for relevant cancer research projects.

The Janus biobank has been run by a number of visionaries and enthusiasts that managed the sample collection and its utilisation in research projects. The resources were limited but the expectations were high that this collection would be of great importance in future research (4). Throughout the collection period the biobank has been supported by strong scientific communities at the Ullevål University Hospital, National Hospital-Rikshospitalet and NRH (from 1 January 2009 organised as Oslo University Hospital). Professor Egil Jellum and biomedical laboratory scientist Anne-Karine Thorsrud as well as chief physician Harald Ørjasæter were key persons in the management of the biologi-

Aage Andersen

Særtrykk av bladet
Mot Kreft 1/87

Janus-prosjektet – tidlig diagnose av kreft?

Blodprøvene fra Janusprosjektet er blitt et ettertraktet forskningsmateriale. Forskere både i Norge og andre land har brukt prøvene i ulike kreftforskningsprosjekter.
– De foreløpige resultatene fra enkelte prosjekter ser lovende ut. Dette dreier seg om kreft i eggstokker, skjoldbruskkjertel og lunger, der forskerne har kunnet påvise forandringene i blodet før sykdommen ble klinisk diagnostisert, sier professor Egil Jellum ved Rikshospitalet.

Overlege Harald Ørjasæter med noen av Janusprøvene som lagres i temperaturer helt ned mot −40°C. De aller fleste av blodets bestanddeler tåler å bli nedfrosset på denne måten.

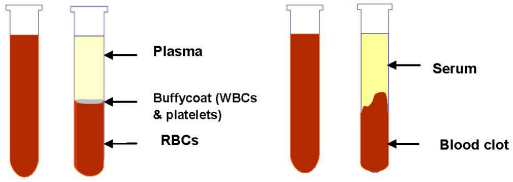
cal samples in the Janus Serum Bank. Former head of research department, Aage Andersen at the CRN was responsible for the data management.

Biobanks and biological material

A biobank can be defined as a systematic collection of human biological material prepared and stored for a certain time, with the information derived from the material's analysis, commonly linked to clinical data. The biological material is typically samples of body fluids or tissues. Storage conditions can vary with respect to temperature, environmental humidity, light exposure and type of vials and caps. With respect to research biobanks, the sample collection can only be used for research purposes, that are considered to be of benefit to the population.

The Janus biobank consists of serum samples that contain small amounts of leukocytes due to incomplete separation. A blood sample in a glass tube will clot after 0.5–1 hours if no anticoagulant is added. Red blood cells (RBC), white blood cells (WBC), and platelets are trapped in the clot separating them from the serum. In contrast to plasma, serum does not contain fibrinogen. Serum consists of approximately 90% water, and normally holds proteins (immunoglobulins transport proteins, enzymes), lipids, hormones, vitamins, minerals, salts and ions, amino acids, water-soluble components, inorganic electrolytes, sugar, carbon dioxide and oxygen. Plasma is obtained from a blood sample to which an anticoagulant (EDTA, heparin or citrate) is added (Figure S1).

Figure S1: Illustration of different blood components



The Janus repository

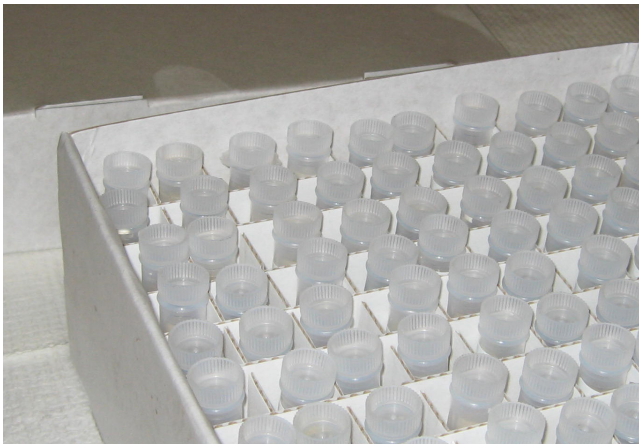
Today the samples in Janus are stored at a commercial freezer department outside Oslo with quality requirements at an ISO certificate level. The storage maintains, a constant temperature of −25 °C. Historically the samples have been located at three different freezer departments in Oslo and the surrounding areas. Parts of the sample collection were stored at −40 °C for a short time. There has been a continuous

Figure S2: Parts of the Janus storage in 2009

work to upgrade the storage conditions. Lately, the old racks have been replaced by new specially designed steel racks (Figure S2).

In the early years of collecting, the Red Cross blood donor samples were stored in bundles of 4–5 vials secured by a rubber band. The samples were placed in boxes but had no specific position. The health examination samples arrived in zip lock bags, marked by county and municipality. During the years all samples have been catalogued in cardboard boxes holding 100 vials each (Figure S3) and stored in racks containing about 150 boxes (64 racks in total). Approximately 90% of the sample collection in Janus is organised in new boxes. The total number of vials in the biobank is 674 386, which includes 452 376 blood draws from 316 951 donors.

Figure S3: Serum sample box with 100 vials



The Janus laboratory

For many years, the Janus laboratory was located at Rikshospitalet in Oslo. Aliquoting of samples in all research projects was performed there up to 2004. The Janus laboratory is today located at the CRN (Figure S4). It is a small laboratory with two workstations. The workbench and ventilation system were upgraded in 2009 into a modern system where ventilation is integrated in the worktop. The serum samples are kept on dry ice during the aliquoting process. Carbon dioxide, which is produced by the dry ice, is effectively removed by the ventilation system. The laboratory fulfils the hygienic and security requirements for biobank operation.

Figure S4: The Janus laboratory at the Cancer Registry of Norway

Biobanks worldwide

The idea of organising biological repositories has existed for many decades. Large-scale collections of human sera have partly been organised on an international level and supported by WHO in 1959 and 1970 (5;6). The International Agency for Research on Cancer (IARC) has published a systematic overview of biological material banks (7). An overview of selected research biobanks worldwide is given in Table S1, and is based on information from the Biobanking and Biomolecular Resources Research infrastructure (BBMRI, http://www.bbmri.eu), and Public Population Project in Genomics (P3G, http://www.p3gobservatory.org). Additional information was gathered from a recent journal article on Nordic biobanks (8). As Table S1 illustrates, the Janus biobank is among the world's oldest and largest biobanks.

In the early days of biobanking the normal storage temperature was between –20 °C to –25 °C. Biobanks established in later years store their specimens at -80 °C and at –196 °C. There has also been a de-

velopment in the type of biological material which is sampled. The older biobanks typically collected serum only. Newer biobanks collect most blood components as well as tissue, urine, breast milk etc. The number of participants varies greatly. Many of the newer biobanks are currently in their enrolment phase. Some biobanks have samples from women only, e.g. The Nordic Maternity Cohorts, the Nurses' Health Study, and The Norwegian Women and Cancer study (NOWAC). Janus has an even distribution of samples from women and men. Many of the biobanks in Table S1 have material from selected groups of the population, e.g. a specific county or age group. Janus has material that is representative to the total Norwegian population, making it particularly well suited for population-based studies.

Table S1: Overview of selected biorepositories worldwide, sorted by year of establishment. Approximate number of participants are given

Biobank	Number of participants	Material	Storage temperature	Country	Established
Swedish Institute of Infectious Disease Control (SIIDC)	358000	Serum	−20 °C	Sweden	1957
Kaiser permanente multiphasic health checkup cohort	150000	Serum	−23 °C / −40 °C	USA	1964
Finnish Mobile Clinic Health Examination Survey	51000	Serum		Finland	1966
Reykjavik Study	30000	Serum	−20 °C	Iceland	1967
FINRISK	23000	Serum, DNA	−70 °C	Finland	1972
JANUS Serum Bank	317000	Serum	−25 °C	Norway	1973
Tromsø study	40000	Serum, plasma, urine, EDTA blood, DNA, hair	−20 °C / −70 °C	Norway	1974
Malmö Preventive Medicine	33000	Plasma, serum	−20 °C	Sweden	1974
Northern Sweden Maternity Cohort	86000	Serum		Sweden	1975
Nurses' health study	238000	Serum, urine		USA	1976
Icelandic Maternity Cohort	48000	Serum		Iceland	1980
Helsinki Heart Study	19000	Serum		Finland	1980
Finnish Maternity Cohort	750000	Serum	−25 °C	Finland	1983
The Nord-Trøndelag health study (HUNT)	100000	Whole blood, DNA, serum, plasma, urine, RNA tubes, cells, buffy coat and Na-heparin tubes	−20 °C / −196 °C	Norway	1984
Alpha-Tocopherol-Beta-Carotene (ATBC) cancer prevention study	30000	Serum		Finland	1984
Västerbotten Intervention Programme (VIP)	95000	Serum		Sweden	1985
Malmö Microbiology	560000	Serum	−25 °C	Sweden	1986
Japan Public Health Center-Based Study	141000	Blood		Japan	1990
NOWAC (The Norwegian Women and Cancer study)	50000	Plasma, DNA, RNA		Norway	1991
Malmö Diet and Cancer	29000	Erythrocytes, granulocytes, mononuclear leucocytes, plasma, serum	−80 °C / −140 °C	Sweden	1991
EPIC (The European Prospective Investigation into Cancer and Nutrition)	522000	Plasma, serum, buffy coat, erythrocytes	−196 °C	10 European countries	1992
CONOR (Cohort of Norway)	200000	Serum, whole blood	−80 °C	Norway	1994
Danish National Birth Cohort	100000	Blood	−20 °C / −196 °C	Denmark	1996
deCode	113000	Blood		Iceland	1996
Million Women Study	1300000	Blood		United Kingdom	1997
Mexico City Prospective Study	160000	Blood	−196 °C	Mexico	1998
The Norwegian Mother and Child Cohort Study (MoBa)	270000	Whole blood, urine , breast milk DNA, RNA	−80 °C / −20 °C	Norway	1999
UK Biobank	120000	Red cells, white cells, plasma, urine	−80 °C / −196 °C	United Kingdom	2000
The Victorian Cancer Biobank	6 000	Tissue, serum, plasma, buffy coat, DNA		Australia	2006

2 Organisation

Ownership

The Janus Serum Bank was owned and sponsored by the Norwegian Cancer Society (NCS) from its establishment in 1973 until 2004. NCS is a national, non-profit voluntary organisation with no state funding, which allocates most of its funds to cancer research. The long-term financial investment from NCS has been of vital importance to Janus Serum Bank. In 2004, the Janus biobank was transferred to the CRN

The Janus Steering Board

Janus has its own steering board, which is responsible for the scientific management of the biobank. Their assignment is to ensure that the use of the bank is in accordance with the license from the Data Inspectorate and the bank's aims, laws and regulations. Furthermore, the board ensures that the applicants that use the bank have the necessary permissions from the Regional Committees for Medical and Health Research Ethics.

The Janus steering board has 7 members from different special fields at institutions and universities across Norway. It consists of at least one member from the Norwegian Cancer Society as well as one member from the CRN. The chairman is elected among and by the members. The composition of the board is approved by the CRN and the term of office is 5 years. The board approves or rejects applications by general majority. Meetings are arranged when necessary but not less than once a year.

The secretariat

The secretariat is located at the CRN, which appoints its members. It is responsible for the daily management of the bank, which includes management of the data material as well as the serum samples. It coordinates all research projects and is in continuous and close dialogue with the principal investigators, IT personnel and laboratories.

Regulations and licence

In the early 1990s, it was determined that the Janus Serum Bank became subject to the Personal Data Filing System Act. In 1993, the CRN received a license to establish a personal data filing register of the Janus donors. The license was in the CRN's name, since all the data were stored there. The license was renewed in 2009 and is valid through 2018.

During the bank's first years, the donors gave their consent for their samples to be used for "cancer research". The broad consent is not valid according to today's laws. Samples collected in 1997 and onwards are collected based on a voluntary and informed consent (Act relating to biobanks, § 12). However, the Data Inspectorate has approved the use of data and biological samples collected in the period 1973–2004. The donors are free to unconditionally withdraw their consent at any time, and their samples and data will then be deleted (Act relating to biobanks, § 14).

Use of biological materials and the data attached to them is subject to strict ethical and legal restrictions. An ethical framework for previously-collected biobank samples and data has been discussed in several previous articles (9-12). Interestingly, thorough ethical analyses of the advantages and disadvantages of different informed consent practises, regarding how they respect autonomy and integrity, have resulted in recommendations for broad consent practices that are rather similar to the original informed consent used in the Janus biobank (9).

How to apply for serum samples from the Janus Serum Bank

On the Janus Internet site:

http://www.kreftregisteret.no/janus

a standardised application form is available. The formhas to be filled in and submitted together with a scientific project protocol. The protocol should include the background and aims of the study, description of the methods, arguments for the serum volume, power calculations, outline of the project finances, and a list of planned publications. At least one collaborator/co-author from the CRN and/or the Janus Serum Bank has to be appointed. Applications are evaluated by the Janus Steering Board within 1-2 months. All research projects have to be approved by the Regional Committees for Medical and Health Research Ethics prior to sample delivery. Applicants outside Norway require a Norwegian collaborator to coordinate the application process.

3 Characteristics of the Janus cohort

Cohort members

The Janus cohort members consist of three subgroups of donors: I) persons undergoing routine health examination in different counties in Norway, II) Red Cross blood donors from Oslo and surrounding areas, and III) previous Janus donors referred to the NRH for cancer treatment, where samples are collected before treatment starts. In the following text, most tables and figures distinguish between these three subgroups. The members of subgroup I) filled out a questionnaire on risk factors for cardiovascular disease, e.g. smoking habits, alcohol use and body-mass index. The questionnaire data are administered by the Norwegian Institute of Public Health. No such information was collected for subgroup II.

The Janus register has the following information about the donors: name, day, month, year of birth, PID, gender and county of residency at the time of blood draw. The total number of donors in the Janus Serum Bank with existing samples is 316 951 (Table S2). Less than 1% (3080) of the donors have not been identified due to invalid PIDs. For a small proportion of the donors (1786) the samples are missing mostly due to serum consumption in different research projects and minor accidental spills. The average age at enrollment for all donors is 41 years. The Red Cross blood donor group (average age 34) is younger than the health examination group (average age 42). The reason is that the donors in the Oslo investigation in 1982–87 and in the health examination from 1985–1999 were in their 40s. Donors from the health examinations constitute approximately 91 % of the cohort and Red Cross blood donors about 9% (Table S3). Post-diagnostic samples are available for 3316 Janus donors (subgroup III). A total of 7473 donors have contributed both as health examination donors and Red Cross blood donors. Table S3 shows the number of donors in the Janus cohort by sex and sample origin. The number of male donors is slightly higher than that of female donors, 52% and 48% respectively, because the Oslo investigation in 1982–87 included men only. In addition, the proportion of men among blood donors is higher than that of women in the general population.

Table S2:
Number of donors included in the Janus Serum Bank

Persons	Number
Janus cohort	321817
Invalid PID	3080
Missing samples	1786
Total included	316951

Table S3:
Number of men and women in the Janus cohort

Sample origin	Men	Women	Total
Health examinations	150779	138310	289089
Red Cross blood donors	17438	14581	32019
NRH	1494	1822	3316
Donors in several categories	4321	3152	7473
All donors	165390	151561	316951

By 31 December 2008, 85% of donors were still alive, 14% were dead and 1% had emigrated (Table S4). The vital status distribution is similar for the health examination and Red Cross subcohorts. Among donors with post-diagnostic samples the death rate is 46.5%.

Description of the sampling procedures

The Janus database has the following information about the samples: day, month, year of collection, position at the repository and county at blood collection. Remaining sample volume is being registered for every sample that is thawed in connection with a project.

The volume of serum samples collected from health examination donors ranged from 1 to 10 ml, and from one to a maximum of four consecutive samples were collected. In the period 1973–1991, the Red Cross blood donors gave a sample of 50 ml to the Janus Serum Bank. In the period 1997–2005, the volume was reduced to 20 ml. The serum was aliquoted into 5-ml polypropylene vials and frozen. Samples from young blood donors (25–30 years of age) were collected at 3–5 year intervals, whereas in the case of the older donors (>40 years) samples were collected more frequently. Thus, from certain individuals, the serum bank may contain up to 17 consecutive blood samples collected since 1973, whereas in other cases only one to three samples are available (13). Consecutive serial samples from the same donors are of particular interest for validation of whether the biomarkers under study are sufficiently stable over time, to allow meaningful analysis.

As shown in Table S5 the largest proportion of the Janus donors has donated serum only once (239 279 donors). More than 42 500 individuals have donated

Table S4: Vital status of the Janus donors

Sample origin	Alive		Dead		Emigrated	
	No	%	No	%	No	%
All donors	269182	85.0	44446	14.0	3323	1.0
Health examinations	245299	85.0	41224	14.0	2566	1.0
Red Cross blood donors	27504	86.0	3703	11.5	812	2.5
NRH	1764	53.0	1537	46.5	15	0.5

twice and more than 24 000 three times. A smaller proportion have donated 4 times (4674) and 6043 individuals have donated 5 times or more.

The Janus biobank is nationwide with samples collected from 17 of the 19 counties in Norway, representing both urban and rural areas (Figure S5).

Table S6 gives the number of donors and number of samples collected in the health examinations only. The only two counties that are not represented are Buskerud and Hordaland. Donors from Oppland constitute the largest part of the Janus cohort with 26.7 %, followed by Finnmark (10.9 %), Sogn og Fjordane (10.6 %) and

Table S5: Number of donors with multiple samples in the Janus Serum Bank

Number of samples	All donors	Health examinations	Red Cross blood donors	NRH
1	239279	233520	13024	3175
2	42574	34204	6788	87
3	24381	20181	3962	26
4	4674	1183	2799	20
5+	6043	1	5446	8

Figure S5: Geographical distribution of sample collection in the Janus Serum Bank

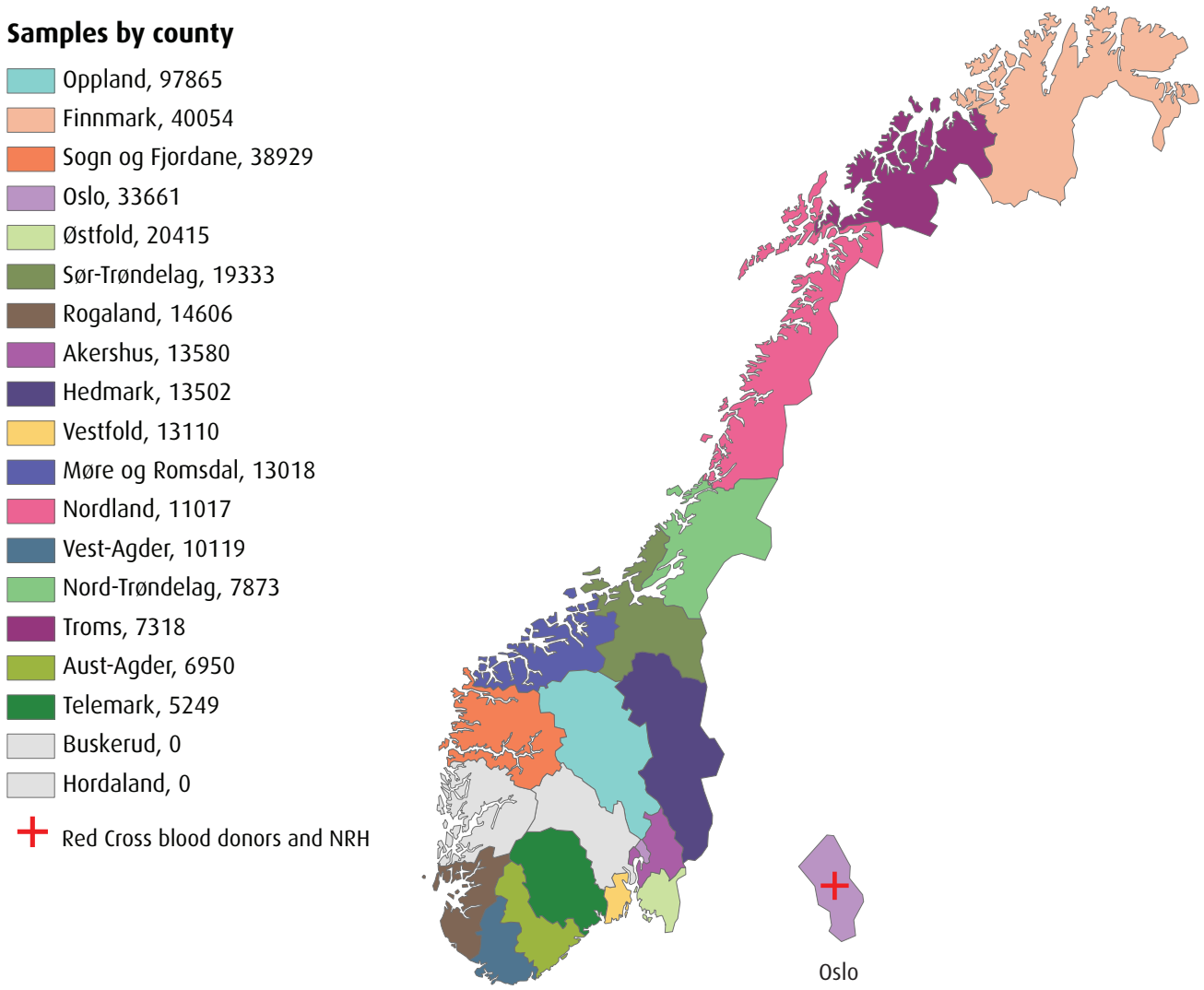


Table S6: Number of samples and donors in the health examinations, by county and gender

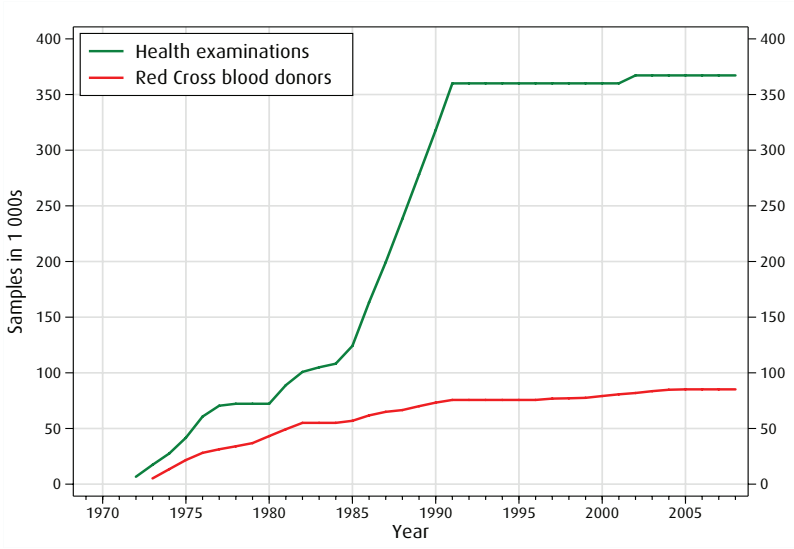
		Men			Women			Total		
No	County	Samples	Percent	Donors	Samples	Percent	Donors	Samples	Percent	Donors
01	Østfold	10068	5.3	9259	10347	5.8	9908	20415	5.6	19167
02	Akershus	6455	3.4	6455	7125	4.0	7125	13580	3.7	13580
03	Oslo	25310	13.3	25077	8351	4.7	8346	33661	9.2	33423
04	Hedmark	6646	3.5	6646	6856	3.9	6856	13502	3.7	13502
05	Oppland	48064	25.3	25395	49801	28.1	26108	97865	26.7	51503
07	Vestfold	6197	3.3	6197	6913	3.9	6913	13110	3.6	13110
08	Telemark	2512	1.3	2512	2737	1.5	2737	5249	1.4	5249
09	Aust-Agder	3445	1.8	3158	3505	2.0	3328	6950	1.9	6486
10	Vest-Agder	4938	2.6	4529	5181	2.9	4881	10119	2.8	9410
11	Rogaland	7051	3.7	6887	7555	4.3	7379	14606	4.0	14266
14	Sogn og Fjordane	19739	10.4	14291	19190	10.8	13726	38929	10.6	28017
15	Møre og Romsdal	6245	3.3	6245	6773	3.8	6773	13018	3.5	13018
16	Sør-Trøndelag	9598	5.1	8688	9735	5.5	9139	19333	5.3	17827
17	Nord-Trøndelag	3920	2.1	3920	3953	2.2	3953	7873	2.1	7873
18	Nordland	5489	2.9	5489	5528	3.1	5528	11017	3.0	11017
19	Troms	3525	1.9	3524	3793	2.1	3793	7318	2.0	7317
20	Finnmark	20128	10.6	12587	19926	11.2	11910	40054	10.9	24497
21	Svalbard	386	0.2	386	223	0.1	223	609	0.2	609
01-21	Total	**189716**	**100.0**	**151245**	**177492**	**100.0**	**138626**	**367208**	**100.0**	**289871***

* 782 persons are included in more than one county

Oslo (9.2 %). A total of 609 samples are from Svalbard inhabitants (a region of Norway located in the Arctic Ocean). The gender specific distribution of samples is fairly similar, except for Oslo, because the collection in 1982–1987 was restricted to men. Table S6 further demonstrates that for the counties Oppland, Sogn og Fjordane and Finnmark, there is a large difference in the number of samples and number of donors, indicating the consecutive collections in these counties.

The serum samples for subgroup I and II were collected in the period 1972–2004. The largest proportion was collected in 1984–1991, especially among health examination donors. In that period the number of samples increased from 110 000 to more than 350 000. Accumulated numbers of samples for both groups are given in Figure S6. Subgroup I and II constitute 367 208 and 85 168 samples respectively. The only serum samples that are still collected by the Janus biobank are samples from the NRH (subgroup III)

Figure S6 Accumulated number of samples collected in different time periods



4 Follow-up of the Janus cohort

Once a year the Janus cohort is linked to the CRN by PIDs, to identify new cancer cases. The CRN has since 1953, systematically collected notifications of cancer for the Norwegian population. A description of the data sources and registration routines at the Registry is given in the first part of this report. The Registry is considered to capture close to 100% of new cancer cases yearly, and this and other quality aspects have recently been evaluated (14-16). The linkages of cancer registry data with the biobank database, create a study base of samples and data, with long-term follow-up and a large number of cancer cases among the donors. The PID, established in the 1960s, allows linkage of data from different sources and to follow a person from early life until a cancer diagnosis, death or other loss to follow-up (emigration or retraction of consent).

The cohort members in Janus are followed up for the event of cancer in the period 1953 to the end of 2008, or until date of death or emigration. As shown in Figure S6 the largest proportion of donors entered the cohort in the late-1980s. The Lexis diagram in Table S7 shows the distribution of person-years at risk of cancer outcome in the different age groups and calendar periods for the Janus cohort members. The total number of person years in Janus is 7 094 753. The age-period-cohort model is a descriptive tool for analysing observations from a Lexis diagram, typically from cancer registries or other disease registries. The model describes rates as a product of age, period and cohort effects (17).

The number of cases has increased year by year since 1978, the largest increase in the period 1998–2008. The baseline samples in Janus were from presumably healthy individuals. However, as shown in Figure S7 some cases were diagnosed before they entered the Janus cohort in 1972. This group comprises approximately 600 cases that were diagnosed with cancer during the period 1953–1971, thus their samples might be affected by diagnostic and treatment parameters.

Cancer incidence

By 31 December 2008 the number of incident cancer cases in Janus was 48 076, 44 578 among health examination donors and 4030 among Red Cross blood donors. Some donors have participated in both subgroups and are therefore counted as cancer cases in both. Of all cases, 3396 have donated post-diagnostic samples at the NRH. Tables S8 and S9 provide an overview of the number of cancer cases in Janus by site and

Table S7: Person years of the Janus cohort members by age group and calendar period (Lexis diagram)

Age	1972-1974	1975-1979	1980-1984	1985-1989	1990-1994	1995-1999	2000-2004	2005-2008	Total
85+				5	109	367	795	3015	**4291**
80-84				36	590	1109	9528	40015	**51278**
74-79			29	558	1503	12171	67679	67226	**149166**
70-74		34	596	1565	14209	79451	96520	64690	**257065**
65-69	29	644	1687	7311	87012	107477	88259	80580	**372999**
60-64	248	1774	6803	58884	114715	93712	117666	294640	**688442**
55-59	472	6969	61148	117888	97741	119925	429323	443096	**1276562**
50-54	4346	61933	118704	98160	122843	437175	501731	60317	**1405209**
45-49	15998	101310	94861	119112	443615	507415	68706	26128	**1377145**
40-44	11837	73322	86651	258867	461975	65795	28725	14049	**1001221**
35-39	2898	54578	40007	48952	45770	25267	13594	5709	**236775**
30-34	2833	29240	36182	35408	25086	12064	5629	3112	**149554**
25-29	3887	28560	24338	16349	11693	3254	1363	768	**90212**
20-24	2596	15622	6148	4701	2926	399	261	97	**32750**
15-19	236	722	130	623	353	16	3	1	**2084**
Total	**45380**	**374708**	**477284**	**768419**	**1430140**	**1465597**	**1429782**	**1103443**	**7094753**

Calendar period

sample origin for men and women respectively.

Cancer in male donors

The total number of cancer cases in male donors is 26 216 (Table S8). A total of 24 429 cases are identified among subgroup I, and 2215 in subgroup II. Cancer of the prostate, lung and colon constitute almost half of the cancer burden. Post-diagnostic samples have been donated by 1606 cases at the NRH. These samples are mainly represented by patients with colorectal cancer, lung cancer, melanoma of the skin, prostate cancer and Non-Hodgkin's lymphoma. Figure S8 identifies the cancer sites that contribute substantially to the disease burden in all male Janus donors.

The most common cancer sites in both subgroups combined are prostate cancer, with some 6521 cases, followed by lung, colon, bladder and melanoma of the skin, with 2 974 2 039, 1861 and 1550 cases, respectively. Figure S8 illustrates that there is a relatively high number of rarer cancer sites in Janus, some 632 cases of pancreatic cancer, 393 multiple myeloma cases, 121 liver, 114 mesotheliomas and 57 nasal cancer cases.

Cancer in female donors

The total number of cancer cases in female donors is 21 860 (Table S9). A total of 20 149 cases are identified in subgroup I, and 1815 in subgroup II. Cancers of the breast, lung and female genital organs represent more than half of the female cancer burden. Post-diagnostic samples have been donated by 1790 cases at the NRH. The most common cancer sites in post-diagnostic donors are colorectal cancer, lung, breast and ovarian cancer.

Figure S8 identifies those cancer sites that contribute substantially to the disease burden in female Janus donors. The most common cancer site in both subgroups combined is breast cancer with 6407 cases, followed by cancers of genital organs, colon, lung and melanoma of the skin, with 3967, 1 555, 1495 and 1335, cases respectively. Rare cancer sites like pancreatic cancer, multiple myelomas, liver, mesotheliomas and nasal cancer cases constitute 412, 210, 43, 16 and 25 cases respectively.

Figure S7: Number of cancer cases by year of diagnosis

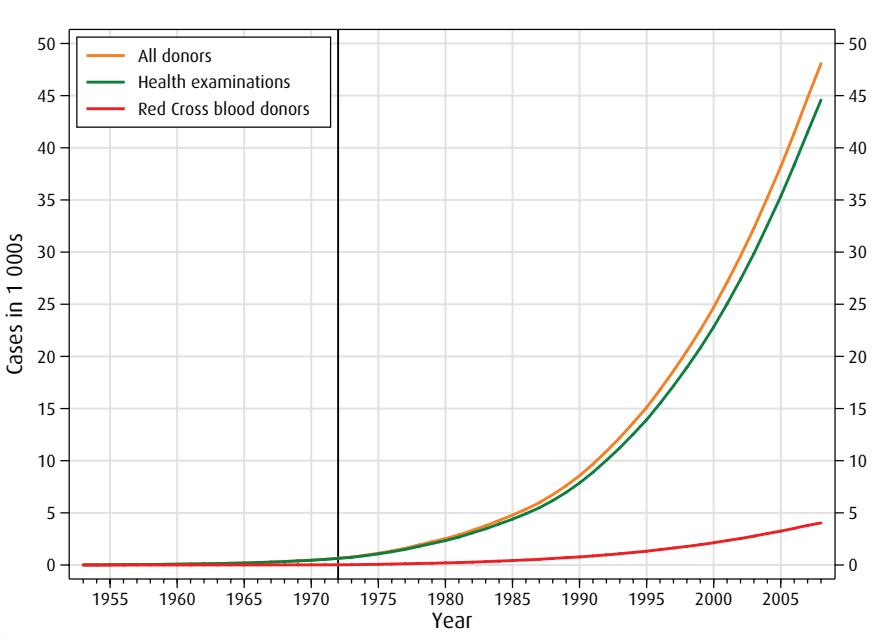


Figure S8: Proportion of common and rare cancer cases among male and female donors

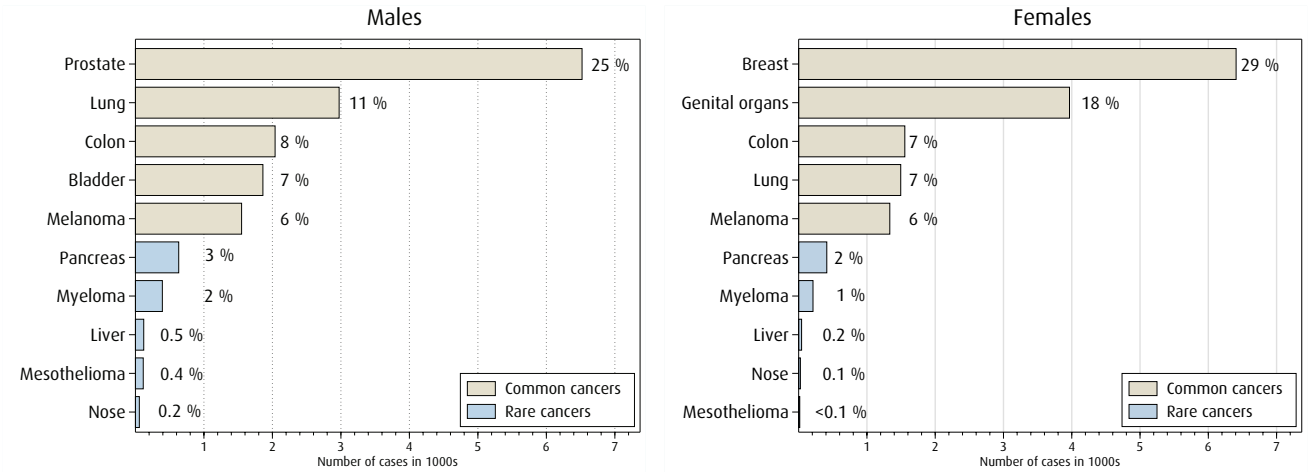


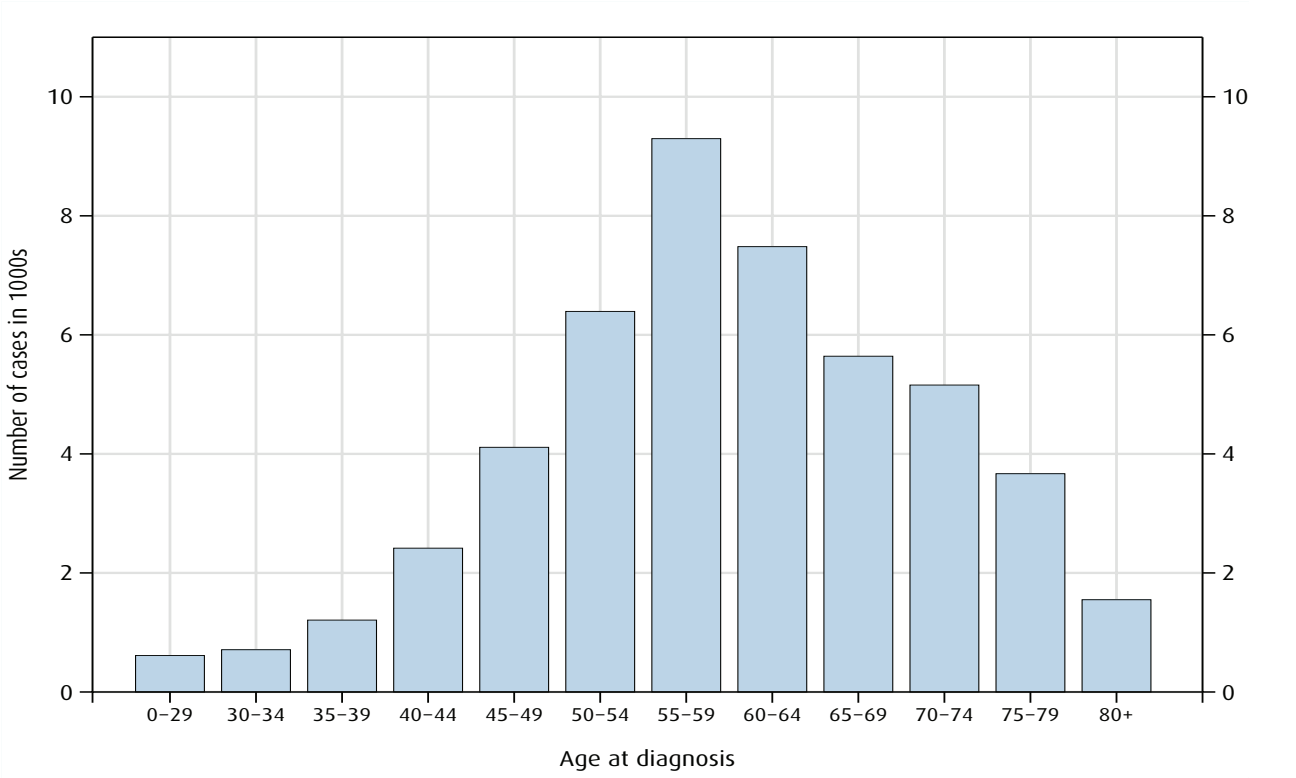
Table S8: Number of incident cancer cases in male donors. Follow up 1953–2008. Cases with post-diagnostic samples at the Norwegian Radium Hospital (NRH) are listed in the last column

ICDO	Site	All donors	Health examinations	Red Cross blood donors	NRH
C00-96	**All sites**	**26216**	**24429**	**2215**	**1606**
C00-14	**Mouth, pharynx**	**687**	**645**	**53**	**59**
C00	Lip	159	153	9	9
C01-02	Tongue	144	131	16	15
C03-06	Mouth, other	130	123	7	12
C07-08	Salivary glands	56	55	1	1
C09-14	Pharynx	198	183	20	22
C15-26	**Digestive organs**	**5625**	**5267**	**440**	**236**
C15	Oesophagus	273	250	27	39
C16	Stomach	846	810	42	30
C17	Small intestine	106	94	14	2
C18	Colon	2039	1901	165	56
C19-21	Rectum, rectosigmoid, anus	1427	1327	122	96
C22	Liver	121	113	8	1
C23-24	Gallbladder, bile ducts	114	108	9	2
C25	Pancreas	632	602	48	5
C26	Other digestive organs	67	62	5	5
C30-34, C38	**Respiratory organs**	**3306**	**3130**	**217**	**223**
C30-31	Nose, sinuses	57	52	6	10
C32	Larynx, epiglottis	250	241	12	15
C33-34	Lung, trachea	2974	2814	197	196
C38	Mediastinum, pleura (non-mesothelioma)	25	23	2	2
C40-41	**Bone**	**39**	**38**	**2**	**9**
C43	**Melanoma of the skin**	**1550**	**1413**	**175**	**93**
C44	**Skin, non-melanoma**	**891**	**797**	**110**	**38**
C45	**Mesothelioma**	**114**	**108**	**10**	**9**
C46	**Kaposi's sarcoma**	**9**	**7**	**3**	**0**
C47	**Autonomic nervous system**	**16**	**15**	**1**	**0**
C48-49	**Soft tissues**	**129**	**120**	**8**	**26**
C50	**Breast**	**29**	**27**	**3**	**3**
C60-63	**Male genital organs**	**7085**	**6584**	**627**	**533**
C61	Prostate	6521	6059	586	477
C62	Testis	467	433	38	38
C60, C63	Other male genital	97	92	3	18
C64-68	**Urinary organs**	**2735**	**2565**	**215**	**119**
C64	Kidney excl. renal pelvis	784	739	58	34
C65	Renal pelvis	90	84	8	3
C66-68	Bladder, ureter, urethra	1861	1742	149	82
C69	**Eye**	**90**	**81**	**10**	**1**
C70-72, D42-43	**Central nervous system**	**898**	**826**	**95**	**57**
C73	**Thyroid gland**	**200**	**185**	**15**	**21**
C37, C74-75	**Other endocrine glands**	**204**	**189**	**18**	**6**
C39, C76, C80	**Other or unspecified**	**438**	**417**	**27**	**12**
C81-96	**Lymphoid and haematopoietic tissue**	**2171**	**2015**	**186**	**161**
C81	Hodgkin lymphoma	138	127	11	15
C82-85, C96	Non-Hodgkin lymphoma	1033	954	94	110
C88	Malignant immunoproliferative diseases	52	51	1	1
C90	Multiple myeloma	393	372	30	18
C91-95	Leukaemia	555	511	50	17

Table S9: Number of incident cancer cases in female donors. Follow up 1953–2008. Cases with post-diagnostic samples at the Norwegian Radium Hospital (NRH) are listed in the last column

ICDO	Site	All donors	Health examinations	Red Cross blood donors	NRH
C00-96	All sites	**21860**	**20149**	**1815**	**1790**
C00-14	Mouth, pharynx	**260**	**237**	**25**	**22**
C00	Lip	61	59	2	2
C01-02	Tongue	54	48	6	6
C03-06	Mouth, other	39	36	3	3
C07-08	Salivary glands	49	44	7	6
C09-14	Pharynx	57	50	7	5
C15-26	**Digestive organs**	**3528**	**3260**	**279**	**188**
C15	Oesophagus	67	61	7	3
C16	Stomach	350	330	20	9
C17	Small intestine	66	61	5	3
C18	Colon	1555	1442	121	65
C19-21	Rectum, rectosigmoid, anus	874	803	69	100
C22	Liver	43	42	1	0
C23-24	Gallbladder, bile ducts	113	104	9	0
C25	Pancreas	412	373	43	6
C26	Other digestive organs	48	44	4	2
C30-34, C38	**Respiratory organs**	**1572**	**1459**	**120**	**128**
C30-31	Nose, sinuses	25	23	2	2
C32	Larynx, epiglottis	42	40	2	5
C33-34	Lung, trachea	1495	1387	115	121
C38	Mediastinum, pleura (non-mesothelioma)	10	9	1	0
C40-41	**Bone**	**41**	**38**	**3**	**6**
C43	**Melanoma of the skin**	**1335**	**1224**	**120**	**46**
C44	**Skin, non-melanoma**	**429**	**389**	**42**	**16**
C45	**Mesothelioma**	**16**	**14**	**2**	**2**
C46	**Kaposi's sarcoma**	**3**	**3**		**0**
C47	**Autonomic nervous system**	**10**	**8**	**2**	**0**
C48-49	**Soft tissues**	**146**	**132**	**15**	**27**
C50	**Breast**	**6407**	**5847**	**621**	**429**
C51-58	**Female genital organs**	**3967**	**3685**	**275**	**679**
C53	Cervix uteri	1004	931	75	116
C54	Corpus uteri	1504	1411	96	220
C55	Uterus, other	13	12	1	0
C56	Ovary	1217	1126	81	285
C51-52, C57	Other female genital	216	194	20	58
C58	Placenta	13	11	2	0
C64-68	**Urinary organs**	**873**	**802**	**72**	**49**
C64	Kidney excl. renal pelvis	371	346	25	20
C65	Renal pelvis	41	36	5	2
C66-68	Bladder, ureter, urethra	461	420	42	27
C69	**Eye**	**66**	**64**	**3**	**3**
C70-72, D42-43	**Central nervous system**	**897**	**845**	**59**	**41**
C73	**Thyroid gland**	**492**	**471**	**24**	**34**
C37, C74-75	**Other endocrine glands**	**171**	**154**	**17**	**3**
C39, C76, C80	**Other or unspecified**	**336**	**298**	**40**	**29**
C81-96	**Lymphoid and haematopoietic tissue**	**1311**	**1219**	**96**	**88**
C81	Hodgkin lymphoma	107	98	8	5
C82-85, C96	Non-Hodgkin lymphoma	668	628	42	72
C88	Malignant immunoproliferative diseases	18	15	3	0
C90	Multiple myeloma	210	193	17	9
C91-95	Leukaemia	308	285	26	2

Figure S9: Age at diagnosis for all cancer cases in Janus



The most common cancer sites in both sexes are in accordance with the profile of cancer seen in the general Norwegian population.

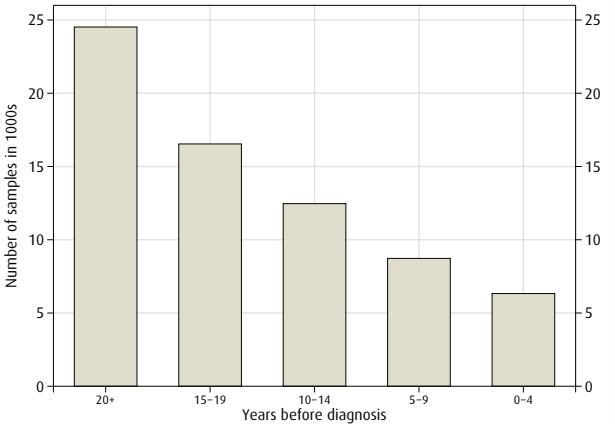
Age distribution and lag time

The largest proportion of cancer cases in Janus are diagnosed in the age group 55–59 years (Figure S9). In the general Norwegian population the age-specific incidence rates per 100 000 person-years are highest in the oldest age groups (80–84, 85+) for all cancer sites combined (see the first part of this report). One-third of all new cancer cases occur among elderly men and women (aged 75 or over). Figure S6 illustrates that the largest proportion of samples in Janus were donated in the late-1980s and early-1990s. Thus, this proportion of the cohort is generally not yet old enough to develop cancer, and this fact suggests that the number of new cancer cases in Janus will continue to increase in the future.

Pre-diagnostic samples are valuable in identifying biomarkers for early detection of cancer and to investigate possible associations between lifetime exposures and risk of cancer. The time between the initiation of disease and cancer diagnosis is the time window where novel markers of early diagnosis can be used (18). The time between sample collection and cancer diagnosis (lag time) for all cancer cases in Janus is given in Figure S10. The largest proportion of the samples has a lag time of either 20 years or more (35%) or 15–19 years (25%). Approximately 10% of the samples that were collected had a lag time of 0–4 years. The rest of the samples material distributes in the lag time categories 10–14 years and 5–9 years.

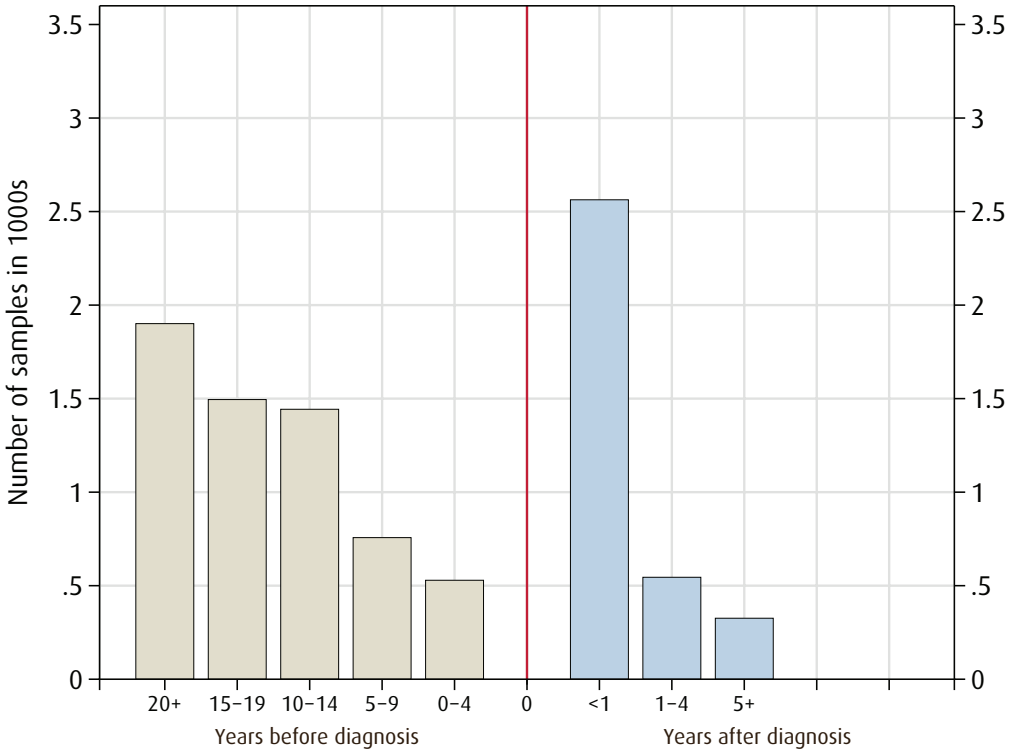
For subgroup III, serum samples collected both before and after cancer diagnosis are available. For those patients ever treated at the NRH, the percentage distribution in lagtime for pre-diagnostic samples is almost similar to that for all patients combined (Figure S11). For post-diagnostic samples, the predominant proportion is collected within one year after diagnosis. As the figure indicates, there are also some samples

Figure S10: Time between sample collection and cancer diagnosis (lag time) in all cancer cases in Janus.



that have been collected 1–4 years or even more than 5 years after diagnosis. Those samples probably originate from the minor number of Janus donors that were recruited to the cohort even though they were diagnosed with cancer before 1972. Alternatively, they might have been treated in other hospitals in Norway before they were referred to the NRH. A third possibility is that they might have been diagnosed with a second cancer. Descriptive analyses of selected sites (cancers of the prostate, breast, colon, lung and melanoma of the skin) showed that the cases with samples collected both before and after diagnosis are limited but adequate for use in research projects (numbers not shown).

Figure S11: Number of samples collected pre- and post-diagnostic, in Janus donors ever treated at the NRH.



5 Quality assurance

Quality assurance (QA) in a biobank is the process of verifying or determining whether all procedures meet or exceed defined quality goals. The process considers standard operation procedures for the biobank sample, from donor to research laboratory, covering sampling, transport, storage, tubes and caps, aliquoting samples and shipment. Monitoring and documentation at every step may reduce pre-analytical errors. Two key principles characterise QA: I. Suitable for intended purpose, and II. Correct handling first time.

I. Suitable for intended purpose

The potential value of a biobank depends on the quality of the samples, i.e. to what extent they may reflect the biological, or biochemical situation in the individual at the time of sampling. Storage conditions can vary with respect to temperature, environmental humidity, light exposure, and type of vials and caps. Most errors in laboratory results are due to pre-analytical factors (sampling routines), and have been estimated to constitute about two-thirds of all errors (19). The sample quality is essential for obtaining reliable measurements of archival samples, and lack of component stability may invalidate research results. Several serum components have been investigated in stability studies in the Janus Serum Bank.

Samples from health examinations

The first health examination was carried out in Oslo 1972–1973 among men. In the period of 1974–1988 the health examination surveys were performed in three counties: Finnmark, Sogn og Fjordane and Oppland. During this first enrollment (1974–1978), iodoacetate was added to all samples to stabilise the blood glucose.

In the period 1985–1999 the health examinations among people in their 40s were performed across the country. The examinations were all subjected to cardiovascular disease and risk factors. Blood sampling was done whenever the subject attended i.e. in a non-fasting or random state. Most women attended early in the morning ; most men attended in the afternoon. In 2002, an examination of health and life conditions in Troms county was completed. The Janus biobank received samples from most of the counties and from several periods in each county. Until 1986, the samples were collected in plain tubes without additives, while the samples from the later years were collected in gel vials. After coagulation at room temperature and centrifugation, the serum was transferred to a new vial (glass or polypropylene tube) before shipment, or sent in a gel tube to Ullevål University Hospital in Oslo for serum analyses. The Janus Serum Bank received the residue volume after the analyses. The samples were shipped in batches of 200–300 in specially constructed cooling containers, which maintained a temperature between +1 °C and +10 °C, and reached the laboratory 45–60 hours after collection (2). Depending on transport distance to Oslo, the freezing of these samples could be delayed for days.

Samples from Red Cross blood donors

The sampling routine of blood donors has been fairly unchanged over time. Samples were collected at daytime between 8 am and 6 pm. The donors were non-fasting; blood was drawn with the donor in a supine position. The tubes had no additives or separating gel; samples were coagulated at room temperature and stored at 4 °C overnight before transport to Janus Serum Bank. The clot time could vary from 14 to 28 hours. After centrifugation the serum was transferred to new vials before freezing. The serum was stored in 5 ml polypropylene tubes. Samples collected in 1973–1975 (about 7000 vials) were lyophilised and have to be rehydrated before use.

Samples from the Norwegian Radium Hospital

Janus donors being hospitalised at NRH are asked to give a sample to Janus before treatment. The samples are collected in tubes without additives. After coagulation at room temperature and centrifugation, the serum is transferred to polypropylene tubes and frozen.

For all three subcohorts, the samples have been stored in paper boxes with lids in a commercial freezer department. Exposure to light has occurred during collecting, retrieval, thawing and aliqouting of samples.

Stability studies

Epidemiological studies may be significantly enhanced by using biochemical analyses of stored blood samples collected from the population being studied. The aim of a clinical laboratory test is to measure the concentration or activity of a component in a body fluid or tissue to give information relevant to a patient's clinical state. This procedure implies that the composition of the samples for analysis should not change during the pre-analytical phase. Stability studies have been performed to describe the quality

and usefulness of the archival serum samples in the Janus Serum Bank.

1. Study of biochemical components in serum, to describe the impact of preanalytical sample handling, short- and long-term storage at –25 °C.

Sodium levels showed a significant difference of +3.9 % in long-term stored serum samples demonstrating only a small degree of sublimation during storage. After short-term storage, the difference in level of potassium (+19.9%) and bilirubin (–32.4%) showed the impact of delayed clot time and light exposure. Calcium, iron, creatinine and uric acid showed non-significant or numerically small differences in the levels for short- and long-term storage (20).

2. Study of long-term stability of protein biomarkers

Levels of albumin, asparagin amino transferase (AST), cystatin C, Immunoglobulins (IgE and IgG), sex hormone binding globulin (SHBG) and transferrin showed non-significant, or numerically small group differences. Large differences after short-term storage were seen for ferritin (–18.5%), alanin amino transferase (ALT) (–41.1%), and creatinin kinase (CK) (–41.1%), while insulin C-peptide showed large differences after long-term storage (–98.7%). The study demonstrated that immunoglobulins which are often analysed in the Janus biobank, are quite robust to long-term storage, while the three-dimensional structure may be critical for enzymes' properties and function and therefore seemed to be more fragile (21).

3 Estimation of folate recovery in long-term stored serum samples

The accuracy of measurement of folate in archival serum samples has been difficult to assess due to degradation during storage and methods offering low recovery. Folate was measured by three methods, microbiological assay, liquid chromatography tandem mass spectrometry (LC-MS/MS) assay and as p-amino benzoyl glutamate (pABG) equivalents giving recoveries of 41.3, 55.7 and 78.9 %, respectively. For assessment of the initial level, folate determined as pABG equivalents gave the most reliable result (22).

A study on hormones focusing on stability and methodology is ongoing in the Janus Serum Bank.

Quality and applicability of DNA

The Janus Serum Bank is not established for genetic analysis purposes; however, the serum samples do contain trace amounts of DNA. These trace amounts are probably due to an incomplete separation of serum from the blood clot.

The quality and amount of the DNA in the Janus Serum Bank have so far not been investigated. Ivarsson et al. (23) have validated genotypes in archival maternal serum samples. The study compared concordance of genotypes between DNA extracted from 200µl whole blood and serum. The results showed a median DNA yield of 15 mg/L (1–34 mg/L) from fresh whole blood and 90 µg/L (0–4800 µg/L) from archival serum samples. Compared to fresh samples from the same women, the archival samples had a DNA yield of 0–14 % decreasing with storage time in the biobank. Sjöholm et al (24) showed that the DNA yield in the archival serum samples could be a useful source of DNA for genetic epidemiologic studies and for reliable studies on genotyping results, provided that at least 0.2 ng of serum-derived DNA is used in the TaqMan genotyping assays (as allelic loss can occur if smaller amounts are analysed). These results should also be valid for the serum material in the Janus biobank.

The Janus serum collection has been used in two genotyping studies: Bjørge et. al (25) examined BRCA1 mutations in ovarian cancer and borderline tumors in Norway, using a nested case-control study design and Ulvik et. al (26) investigated single nucleotide polymorphism (SNP), genotyping in unprocessed whole blood and serum via real-time PCR.

In both studies, 5–7 µl archival serum was used for detection of SNPs by real-time polymerase chain reaction with a success rate of 83.2–97.9%.

The DNA amplifications were performed on a large set of serum samples, n=2 955 (BRCA1 gene), and n=2 500 (MTHF-reductase gene). Both studies demonstrated that amplification of DNA from serum samples is possible.

II. Correct handling first time

Correct handling the first time requires a high degree of accuracy in all parts of the process. The Janus Serum Bank has established an organisation with explicit procedures for quality assurance, including a quality manual listing the instructions that regulate all parts of the biobank body. The quality system is designed to meet the requirements in the ISO/IEC 17025 standard. All equipment is regularly maintained for optimal performance.

Quality assurance of the samples in research projects

The requested samples are transported from the repository to the Janus laboratory in cooling elements and are immediately placed in freezers. All freezers at the Registry have acoustic alarms and are, in addition, connected to a security company in the event of power failure or breakdown. The temperatures are checked and registered daily.

A maximum of 10 samples are thawed and carefully homogenised simultaneously to minimise the time the samples are exposed to light, and remain in the liquid phase. The restriction on the number also minimises the risk of any mix-up of the samples. The identity of the samples is checked at least two times during aliquoting. Only one sample at a time is uncorked to prevent any contamination. The samples are in the liquid phase for approximately half an hour and are immediately placed on dry ice after the requested volume of serum has been extracted. Filtered tips are used for withdrawal of the serum.

Organising the laboratory work

In order to organise the laboratory work Janus Serum Bank is in the process of implementing the Laboratory Information Management System (LIMS) for management of all laboratory processes. LIMS is computer software that is used in the laboratory for the management of samples, users, instruments and other lab functions like workflow and invoice registration. The system will make it possible to keep track of information on the samples, i.e. connection between aliquots and the mother tube, and restrictions on the samples like donor reservations. LIMS will also be utilised for generating picking and shipment lists, for assigning barcodes as well as registration of residual volumes and laboratory results. Security and confidentiality of data are ensured by the use of data systems developed with a very high level of security, including back-up procedures and audit functions that protect against unauthorised access to sensitive data.

Document handling

The Janus biobank has recently started implementing an electronic quality manual system to store concessions, rules and regulations, informed consents, information documents and registration of publications. The programme keeps track of project data like applications, approvals and contracts. The quality manual, changes of standard operating procedures (historical documents), registration of deviation and the internal control document are also stored in this document handling system.

6 How to use the Janus biobank – methodological considerations

All sections in this chapter are based on a peer-reviewed article on the quality assurance of studies linking data from cancer registries and biobanks with an emphasis on the experiences of the Nordic biobanks, such as Janus (18).

Design and methods in research projects

The choice of study design in the relation to the use of Janus depends on the proposed outcome of interest. The most widely used study design is however the nested case-control design. A case-control study is an epidemiological study in which, rather than measuring the experience of an entire population to obtain rates, controls are sampled from the same source population as the cases, and relative rates are estimated. The control group provides an estimate of the exposure distribution in the source population and is a substitute for the denominators of rates or risks (27). The nested case-control study starts with identifying the subjects who have developed the disease of interest during follow-up. For each case, the controls are randomly sampled from those eligible to be controls. The practice of selecting ”best fitting controls” (the controls that best fit the matching criteria) and/or convenience sampling (selecting the controls that are stored adjacent to the case and/or listed next to the cases on sample inventories) (28) is not recommended. When using such a sample of controls, it is not possible to make generalisations about the total cohort as the samples are not drawn at random from the eligible samples in the cohort. The nested case-control design is superior for the study of biomarkers where the measurements may be influenced by analytic batch, long-term storage and/or freeze-thaw cycles.

Another approach is the case-cohort sampling design that allows controls to be selected from a random sample of the whole cohort at the start of the follow-up. The subcohort is representative of the full cohort rather than non-cases. Every person in the source population has the same chance of being included in the subcohort as a control, regardless of how much person-time that person has contributed. All subcohort members at risk at the time of the cases diagnosis serve as controls.

Identification of cases and controls

The Janus donors diagnosed with the disease of interest a sufficiently long time after specimen donation are typically included as cases. The cases are identified by linking the PID in Janus to the CRN. This linkage is performed in every new study since the cancer registry is a dynamic database and the number of new cases among the donors will increase over time.

In some Janus studies on environmental and dietary exposures it might be relevant to select samples from one specific point in time, when the exposures were high and samples from all other time periods are not, due to a lack of significant serum components. However, the most typical approach is to include all cancer cases of a specific site during the follow-up period and select either the first available pre-diagnostic sample of the cases, or the sample closest in time to the diagnosis. Another approach is to select serial samples from each case, where the aim of the study is to investigate prediagnostic changes in biomarkers.

Controls should be selected via random sampling among all those eligible, not just those that appear suitable for matching. A person selected as a control, who remains in the study population at risk after selection, should remain eligible for further selection as a control. Moreover, a person selected as a control whom later develops the disease, and then is selected as a case, should be included in the study both as a control and as a case. Sampling with replacement, which allows a control specimen to occur more than once as a control, is in harmony with incidence density sampling for matched case-control studies (27). In biobank studies, it is common to identify 1–2 substitutes for each control that fulfils the same matching criteria as all controls. Those substitutes will be used if the control specimen cannot be located or the serum volume is too low.

The controls have to be at risk for the outcome, in other words, alive and under follow-up at the time of the case’s diagnosis. Therefore vital status is an important variable. Follow-up should include cancer occurrence, death or emigration.

Matching

Matching refers to the selection of control series with respect to the distribution of potentially confounding factors, and is usually performed as a means of increa-

sing efficiency. Common matching variables in Janus are sex, age, date of diagnosis, date of blood collection , county and batch type. Other relevant matching variables may be storage time and thawing status. Matching on only a limited number of variables has been recommended in a recent paper reviewing Nordic biobank studies (8). Actual sampling dates at the level year-month-day (yyyymmdd), instead of approximate ones, are favorable. For instance seasonal matching may be important in micronutrient studies where detailed information on date is essential in evaluating differences in vitamin levels during the year (29;30).

If the aim of the study is to elaborate upon the disease risk related to exposure in the general population, specimen donors selected by health behavior, risk factors and/or sickness, should be avoided. As population representativeness in practice commonly differs from that intended, estimating standardised incidence ratios for a number of different cancer sites, as a proxy for estimating population representativeness, is highly recommended (8). The Red Cross blood donors in Janus are examples of healthy donors not representative of the general population (31). We therefore recommend matching population-based cases to population-based controls, and blood donor cases to blood donor controls. Since blood donors are supposed to be recruited from the healthiest fraction of the population, their use as controls for population-based cases is likely to result in an overestimation of risk.

Data linkages and logistics

The first step in research projects is to link the Janus cohort members to the CRN to identify cases and controls. This linkage gives information on the cancer diagnosis, sample collection date and the location of the sample at the storage facility. After the retrieval of all samples, a complete list of study codes and the coded biological samples are sent to the research laboratories. When analyses are completed, the code list and the laboratory results are returned to Janus. The Janus secretariat attaches all the information required for the study (e.g. the case-control status) and sends the file to the principal investigator or to a statistical analysis centre directly. It is important to ensure that the released data file does not contain accessory information on subjects at a level of detail such that identification of individual subjects would be possible.

In all biobank research projects an essential aspect of quality is that the laboratory analysis is carried out blinded to avoid potential biases. In the Janus Serum Bank, a code-keeping system has been in routine use for many decades. The code of the case-control status is revealed only after completion of all the laboratory analyses. This is an important measure to prevent any data manipulation. The code-keeping system also ensures a high degree of confidentiality; furthermore, patients' names and PIDs are never disclosed (18).

In a few of the research projects involving Janus, several data sources have been used from different institutions. The definition of a unique study code is then essential and is normally generated by the Janus Secretary. Commonly-linked data on confounding variables are occupation, diet and smoking habits. The PID makes possible the retrieval of data from the Norwegian Institute of Public Health. Those data were collected from the health examination questionnaires from the Oslo investigation, 1972–1973 (25%), and the three-counties file (Oppland, Sogn og Fjordane and Finnmark), 1974–1988 (75%). Examples of data items are smoking habits, height, weight, year and age at height/weight measure, and levels of cholesterol and triglyceride. Another source of information is demographics and census data from Statistics Norway, e.g. number of children, birth year of children, occupation, industry, municipality, community (dense/scattered), population (number of citizens in the municipality), county at birth, education, income, number and date of relocation. In such cases, it is recommended that the list of PIDs is sent to those institutions that have the required data and that they themselves do the linkage to obtain the necessary additional information. If the unique study code is sent from Janus together with the personal identifiers, the external institutions can then release the coded data (without identifiers) directly to the PI. During the linkages, it is important that each person in the study population be assigned a unique code. A flowchart of the study logistics and data sources normally applied in Janus studies is illustrated in Figure S12.

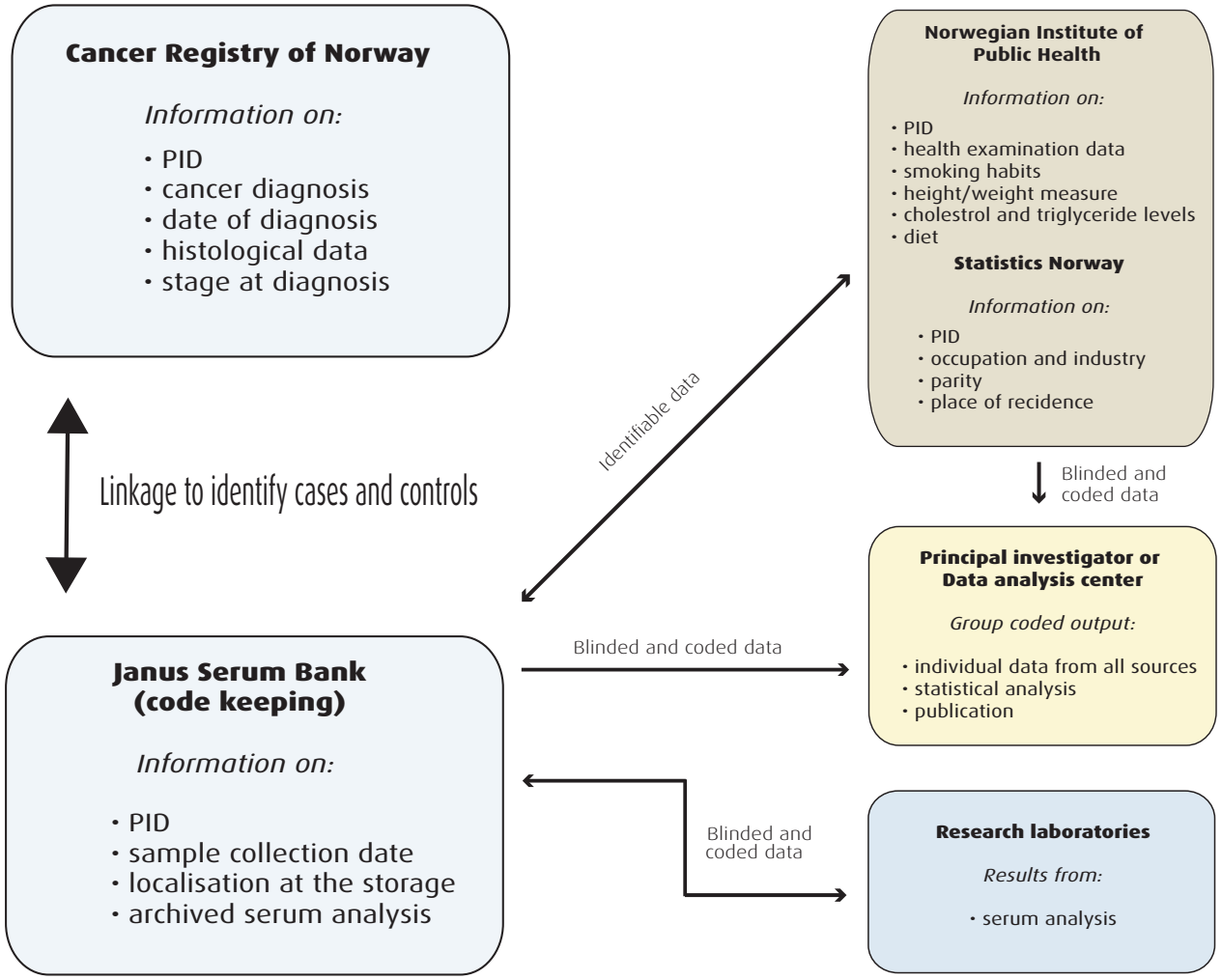
Data management

The writing of an explicit "*Data Management Report*" should become common practice and an important element of the study protocol. This practice is essential for ensuring accuracy in describing the procedures undertaken, particularly when writing scientific papers. The report should contain information on; the identification, loss to follow-up and final numbers of cases and controls; matching criteria; dates of serum sample thawing; missing data and any data errors found. The file record specification should be enclosed as an appendix.

Long-term archiving of the entire study file containing information from all participating biobanks should be organised. The institute responsible for the long-term storage should be stated on the logistics

scheme. The long-term archive file should be based on the unique study code and should not contain any personal identifiers. The code(s) that may be used to link the unique study code to the personal identifiers should be archived at each biobank that originated the samples. It is common that there may be requests for actual crude data for clarifications, new research hypotheses that require further laboratory analyses adjusting for the previously analysed exposures, or requests from collaborators involved in pooling studies that require information from a study published many years earlier. It should be possible to respond to such requests in an efficient manner. Because of the lengthy time spans involved and the requirement for unified data storage formats, it is recommended that the custody of the long-term archive be the responsibility of the host institution. It is therefore sufficient from an ethical and practical point of view that codes enabling linking to personal identifiers remain in the biobank.

Figure S12: Flowchart of common study logistics and data sources in Janus research projects



7 Research projects and important findings

Figure S13: Key scientific findings from studies involving the Janus biobank

Association between different types of Human papillomavirus (HPV) and cervical cancer, other anogenital and oropharyngeal cancers

Association between infection with the bacteria Helicobacter pylori and gastric cancer

Association between elevated levels of Prostate-specific antigen (PSA) and development of prostate cancer

Association between elevated serum CA 125 levels and ovarian cancer

The Janus biobank has since the 1980s been utilised in a numerous research projects within the field of cancer epidemiology. The most common hypothesis have involved the investigation of associations between cancer and infections, environmental exposures and lifestyle-related factors. Another important aim has been to investigate biomarkers for the early detection of cancer. Studies on other disease outcomes than cancer have been limited (32). Figure S13 highlights some important results from aetiological studies in Janus that have had implications on the treatment regimes of cancer patients. The studies reported associations between the human papilloma virus (HPV) and cervical cancer (33), Helicobacter pylori (H. pylori) infection and gastric cancer (34), as well as elevated levels of Prostate Specific Antigen (35) and cancer antigen 125 (CA-125) and the development of prostate and ovarian cancer (36) respectively.

A common technique used to analyse metabolic or immunological changes in serum samples from Janus has been Capillary Electrophoresis (CE). CE designed for serum protein analysis was used for early detection of the monoclonal component in myelomatosis (37). The ELISA technique has been used in several immunological studies. Both chromatography and electrophoresis were utilised to study pre-diagnostic sera from Janus and to classify certain bacteria (38).

The first scientific paper from Janus was published in 1982. The number of publications has grown over the years, as is shown in Figure S14. In addition, there have been a number of reports, pilot studies and abstracts. Approximately 30% of the projects have been initiated by Norwegian researchers and 70% have been initiated by research groups outside Norway. By 2009 there are more than 15 ongoing projects involving the Janus biobank. An overview of the publications, according to research area, is given in Table S10.

Infections and cancer

A large proportion of the research projects in Janus have focused on investigating the association between infections and cancer, in particular HPV. The strong association between HPV-18 infection and risk of cervical adenocarcinoma was confirmed in a seroepidemiological study (33). Another study found a significant increase in the risk of head and neck cancer among HPV-16 seropositive cases (41). A significant increased risk of developing esophageal cancer was observed in HPV 16-seropositive subjects (42) as well as among women diagnosed with non-cervical anogential cancer, particularly vulvae and vaginal cancers (89). A positive association was found between cancer of the anal and perianal skin and infection with HPV

Figure S14: Accumulated number of publications using the Janus Serum Bank

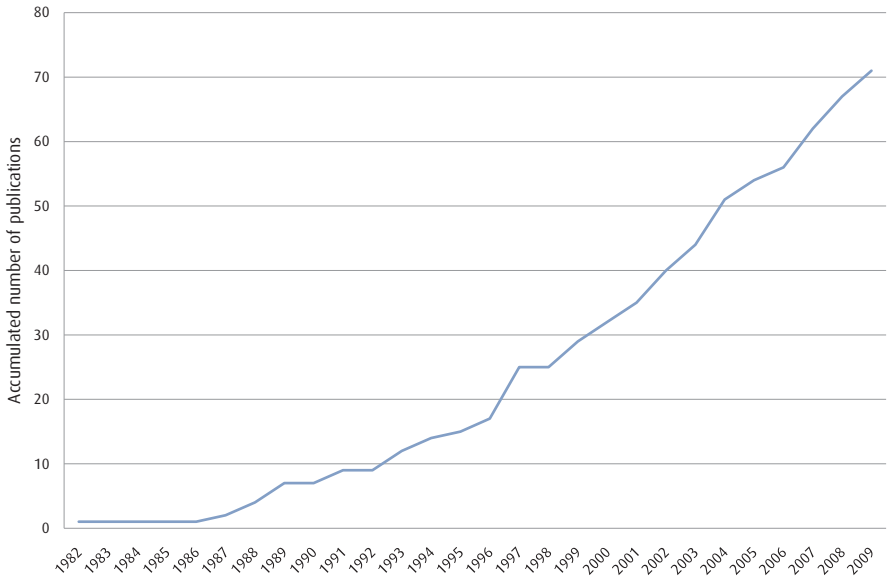


Table S10: Overview of the publications in the Janus biobank, separated by research field

Research field	Biomarker / Biobank	Type of cancer	References
Infections and cancer	HPV	Cervix	(33;39;40)
		Head and Neck	(41)
		Esophagus	(42)
		Prostate	(43)
		Anal and perianal skin	(44)
	HPV DNA	Cervix	(45)
	H. pylori	Gastric	(34;46-48)
	Epstein Barr virus	Non-Hodgkin lymphoma	(49)
		Nasopharyngeal	(50)
	EBV and CMV viruses	Testicular	(51)
	JC polyomavirus	Colorectal	(52)
	SV40 antibodies	Mesotelioma	(53)
	Human herpesvirus 8	Multiple myeloma	(54)
	C. trachomatis	Cervix	(55-57)
	Chlamydia antibodies	Prostate	(58)
	HPV and C. trachomatis	Cervix	(59)
	HPV and C. trachomatis and smoking	Cervix	(60)
Early detection of cancer	PSA	Prostate	(35)
	CA-125	Ovary	(36)
	Androgens	Prostate	(61)
	Epstein Barr virus	Hodgkin lymphoma	(62)
	Thyroglobulin	Thyroid	(63)
	Immune Anti-tumor respons	Lung	(64)
	Mesothelin	Malignant mesothelioma	(65)
	Insulin-like growth factor I and binding protein 3	Breast cancer	(66)
	Sex hormones	Prostate	(67)
	Testosterone	Prostate	(68)
	Vitamin D	Prostate	(69)
Environmental exposures and cancer	Organochlorines	Breast	(70)
		Non-Hogkin lymphoma	(71)
		Testis	(72)
	Selenium	Thyroid	(73)
	DHEA	Thyroid	(74)
Lifestyle related risk factors and cancer	Smoking/Cotinine	Lung	(75)
	Smoking/Cotinine	Cervix	(76)
	Fatty acids	Prostate	(77)
		Thyroid	(78)
		Breast	(79)
	Obesity/lipids	Colon	(80)
	Diet/leptin	Prostate	(81)
	Diet/phytoestrogen	Prostate	(82)
Cancer prognosis	Vitamin D	Prostate	(83)
	Mesothelin	Malignant mesothelioma	(65)
Genetic studies	SNP-analyses	Colorectal	(26)
	BRCA1-mutations	Ovarian	(25)
QA- stability studies	Serum proteins	-	(21)
	Sodium, calcium, iron, creatinine, uric acid, potassium, bilirubin	-	(20)
	Albumin and free fatty acids	-	(84)
Descriptive studies	Janus	-	(4;38;85;86)
	Nordic biobanks	-	(8)
	Nordic biobanks/registries	-	(18)
Methodological stuides	Chromatography and capillary electrophoresis	-	(37;87)
	BRCA1-mutations	-	(88)

-16 and 18 (44). No association was seen between the serologic markers of HPV-16, 18, and 33 infections and the risk of prostate cancer (43).

H. pylori infection and risk of gastric cancer have been investigated in several studies. It was found that gastric (but not non-gastric) non-Hodgkin lymphoma, was strongly associated with previous H. pylori infection(46). A nested case-control study in the Janus cohort concluded that the results strengthen the evidence that H. pylori infection is a risk factor in non-cardia gastric cancer. A negative association with H. pylori infection was found for cardia cancer (47). A recent paper reported two aetiologies of cardia cancer, one associated with H. pylori atrophic gastritis, resembling non-cardia cancer, and the other associated with non-atrophic gastric mucosa, resembling esophageal adenocarcinoma. Serological markers of gastric atrophy may provide the key to determining the gastric versus the esophageal origin of cardia cancer (48).

One landmark study reported a positive association between Chlamydia trachomatis (C. trachomatis) and risk of invasive squamous-cell carcinoma (SCC) of the uterine cervix (55). These results were confirmed in other studies using sera from three Nordic biobanks (56;57). The joint effect of HPV and C. trachomatis and smoking on risk of cervical cancer was investigated in the same Nordic biobanks. Results showed that HPV-16, C. trachomatis and smoking are likely to be risk factors for SCC and jointly, there is strong antagonistic effect (59;60).

Serum from Janus has also been used to investigate the association between the Epstein-Barr virus (EBV) and Hodgkin lymphoma. Results showed that the development of the disease may in some patients be preceded by enhanced activation of EBV. The study could not provide direct answers as to whether EBV has a direct role in the pathogenesis of the disease, or is simply a marker for a more fundamental factor affecting the immune control of latent infection (62). This finding were supported by similar findings for non-Hodgkin lymphoma (49).

Preclinical tumor markers and early detection of cancer

A study on early diagnosis of ovarian cancer on measuring CA-125 levels, provided a new insight into the preclinical biology of ovarian neoplasia (36). Serum CA-125 levels were determined retrospectively for specimens collected from 105 women who subsequently developed ovarian neoplasia, and from 323 matched controls. The distribution of CA-125 levels was significantly different between the case and control populations. Serum thyroglobulin was found to be a preclinical tumor marker in subgroups of thyroid cancer patients in Janus. Results indicated that serum thyroglobulin tends to be elevated years prior to the clinical appearance of thyroid carcinoma, whereas serum-TSH is not (63). Janus was participating in collaborative analysis of 18 prospective studies investigating the association between sex hormones and prostate cancer. No association was found (67).

Environmental exposures and cancer

The sample collection in Janus is particularly suitable for investigating environmental exposures and cancer, since a large number of the samples were obtained at a time when a number of carsinogenic substances were still in use. The three studies that are published on this topic are based on serum samples from the 1970s when organochlorine exposures were high. A positive association between Non-Hodgkin lymphoma and polychlorinated biphenyl (PCB) was shown in one study (71), and between testicular cancer and exposure to dichlorodiphenyldichloroethylene (DDE) and chlordane compounds in another (72). However, no association was shown between breast cancer and organochlorine levels (70). There is also an ongoing project on organochlorines and risk of cancer of nine different sites.

Lifestyle-related risk factors and cancer

Lifestyle-related risk factors like smoking and diet have been investigated in Janus projects with different cancer outcomes. Serum cotinine level as a predictor of lung cancer risk has been investigated in 1741 Janus participants. Mean serum cotinine levels were higher in cases than in controls, and were found to be a predictor of risk of lung cancer among smokers (75). Smoking as an independent risk factor for cervical cancer in women infected with oncogenic HPV was confirmed in a Nordic collaborative study (76). A further study hypothesized that leptin increases the risk of colon cancer, and furthermore that leptin may provide a link between obesity and the disease (80).

QA stability studies

A vital component of the work within the Janus biobank is quality assurance and studies performed to investigate the stability of a number of serum components (electrolytes, minerals, water-soluble molecules, enzymes, immunoglobulines, transport proteins, glycoproteins, peptides and hormones) after long-term storage in the Janus serum bank. The studies showed some sublimation (4%) in the samples after 25 years of storage, and demonstrated that commonly-analysed immunoglobulins are quite robust to long-term

storage, while some enzymes appeared to be more fragile (20;21).

The effect of long-term storage on the concentration of albumin and free fatty acids (FFA) in serum was measured. FFA and albumin could increase in response to several years of storage at –25°C. It was suggested that the storage-time-dependent increase in FFA was due to FFA liberation from lipoprotein triglycerides, whereas the apparent increase in albumin concentration possibly could be attributed to an unfolding of the protein (84).

Genetic studies

Few genetic studies have been performed in Janus. However, two studies that used polymerase chain reaction (PCR) methods were successfull in detecting the polymorphisms of interest. One study found a significantly reduced risk of colorectal cancer in subjects with the MTHFR 677 TT and MTR 2756 GG genotypes (26). Prevalence of BRCA1 mutations in ovarian and borderline tumors was investigated in a case-control study. Four founder mutations were analysed and results showed that mutation carriers have a very high risk of developing ovarian cancer (25).

Methodological studies

Analysis of DNA variations in biological samples most frequently involve PCR performed on extracted genomic DNA. Amplification of DNA from plasma and serum samples has been rare applied. DNA amplification on a large set of serum samples (n=2955) from the Janus collection has also been studied. Of the 11 820 PCRs performed, the overall success rate was 91.3%, which is comparable to the success rate of PCRs performed on genomic DNA (88). The advantage of the method is its ability to utilise archival material stored in serum biobanks for a long period.

8 Strengths and limitations

At the time Janus Serum Bank was launched, few amongst the scientific milieu had strong inclinations as to the profound importance of such materials in future cancer research. The establishment of the Janus Serum Bank was made possible by a huge voluntary and communal effort in Norway. However, the funding was restricted and therefore gave room for only simple logistical solutions, relative to the options available to well-funded and modern biobanks, with respect to temperature monitoring, sample access etc. The Janus biobank is a child of its age carrying both strengths and weaknesses compared to a modern biobank.

The Janus biobank holds a unique position because of its close and longstanding relations to the CRN. Janus has a high number of sera with a follow-up time of up to 35 years, and can thus provide research projects with the required number of cases, even for rarer cancer forms. Sequential samples offer a unique possibility to follow the development of the disease in prediagnostic changes over the years. The biobank contains serum samples from as far back as the early-1970s, a time when dietary and smoking habits in the general population were quite different, and certain environmental pollutants - later established as carcinogenic - were not yet banned from use. These samples have proven to be important in cancer projects investigating the impact of environmental exposures. The presence of some DNA in the Janus samples may offer the possibility for single nucleotide polymorphism (SNP) analysis. Although the quality and amount of the DNA material in Janus Serum Bank have not been investigated, two projects have successfully performed SNP analysis. Two of the obvious benefits of the Janus Serum Bank are that it provides linkage to the national high-quality cancer registry, and that it provides linkage to the deaths registry in order to match vital status, and to the national public health institute for questionnaire data.

Today the Janus Serum Bank performs high-quality operational procedures according to a quality manual that implements the retrieval of the correct sample, transport to the laboratory, thawing, aliquoting, tubes and caps, and shipment.

Documentation is an important part of the quality work, and use of every sample in the bank in an earier project is recorded. The storage has been upgraded and now has a good quality logistic system.

The Janus Serum Bank has a strict code-keeping policy. The code will not be broken before the analyses have been completed and the results have been sent to the Janus Secretary. This policy ensures blind analyses and honest research.

One limitation of the Janus biobank is the undocumented preanalytical sample handling. Samples donated by both blood donors and persons participating in health examinations were collected in a sub-optimal way. Blood donor samples had a prolonged clot time before separation and freezing of serum. For the health examination samples, delayed freezing of the serum samples due to long transport distance may have reduced the quality of the samples. However, studies show no significant changes in the levels of a number of common biological components in rat serum, refrigerated for seven days (90), and in human serum, refrigerated for four days (91). The storage temperature of –25 °C is sub-optimal according to present requirements. Stability studies have shown that the pre-analytical sample handling and the storage temperature have an impact on some serum constituents (20, 21). In the early years of collecting (1973–1978), iodoacetate was added to the samples to stabilise the blood glucose. Iodoacetate may disturb the measurements in some assays (GC-MS of homocystein and double-antibody radioimmunoassay of IgF), and these samples have therefore been excluded in certain projects (3, 4).

In contrast to modern biobanks, Janus does not contain many small aliquots from each sample, but from the blood donors there are 4–5 larger aliquots. The repeated refreezing of the mother tube after aliqouting may decrease stability of some serum components and thus reduce the quality of the sample. However, the majority of the samples in the Janus serum bank have not yet been used in research projects, and therefore have never been thawed.

The number of cancer cases continues to increase in the bank, as does the lag time (the time between date of collection and date of diagnosis) for the cases. A long lag time may be of critical concern in studies looking for biomarkers for early diagnosis of cancer.

Despite the limitations, the bank has proved to have a large research potential. The bank is internationally unique with respect to its research potential given its size, long-term storage of serum samples and its large number of subsequent cancer cases.

9 National and international collaborations

An underlying philosophy of the The Janus Steering Board is that the collection of serum samples should be utilised as much as possible for relevant cancer research projects. Researchers are therefore encouraged to submit proposals for collaborative studies.

Already in 1980 the Janus biobank collaborated with 10 laboratories in Europe and the United States, and more than 1000 serum constituents were analysed in a search for early pre-clinical biochemical changes in sera.

The Janus Serum Bank and the CRN have collaborated with institutions both in Norway and abroad. The pioneering study of H. pylori and gastric cancer was published in collaboration with Ullevål University Hospital in Oslo, and a project on vitamin D associated with cancer has been published in collaboration with Aker Hospital in Oslo. The gene mutation study among ovarian cancer patient in Janus was initiated at the NRH and a project on gene polymorphisms and colon cancer was initiated by the homocysteine group at the University of Bergen.

Through the Nordic Biological Specimen Banks working group on Cancer Causes & Control (NBSBCCC), and the Cancer Control Using Population-based Registries and Biobanks (CCPRB), a large European Union-funded Network of Excellence, the Janus Serum Bank has collaborated with several European institutions. Major research fields has been HPV and different cancer forms and studies on the science of biobanks. Samples have been analysed at the German Cancer Research Center in Heidelberg and at laboratories in Malmö, Sweden and Oxford, England. Most of the statistical work in CCPRB has been performed by the Cancer Registry of Finland. Important projects on lung cancer associated with smoking have been performed in collaboration with IARC, France.

In the U.S., Stanford University, Harvard School of Public Health, Yale University and Johns Hopkins University have been collaborating institutions. A longstanding cooperation with the National Cancer Institute (NCI) in United States has been focusing on associations between environmental exposure (organochlorines) and different cancer outcomes.

10 Summary remarks and future perspectives

Biobanks have become an important resource in cancer epidemiology and have led to an increased interest in aetiological, clinical and gene-environment-interaction studies. The long follow-up time, the nationwide sample collection and the large number of cancer cases make Janus biobank an exceptional resource for modern cancer research. Linking information from biological samples in Janus to the population-based CRN, optimises the biobank's value in cancer research. Janus is an important resource for cancer research and future goals are to optimise the utilisation of the samples, and to improve the accessibility for all national and international research groups focused on cancer as endpoint.

Major scientific goals of the Janus Serum Bank are:

- To motivate studies on rarer cancer forms. The number of cancers such as liver and pancreatic cancer, and multiple myeloma is high in Janus. This feature provides opportunities to set up large epidemiological studies focusing on cancer diseases with poor prognoses, and where the knowledge of aetiology is limited.
- To continue vital quality assurance work to obtain high quality scientific data.
- Initiate new studies examining environmental exposures, in relation to lifestyle-related cancers, and to investigate the effect of changing exposure over time.
- To utilise the subcohort with both pre- and post-diagnostic serum samples in studies on cancer treatment and prognosis.
- To focus on site-specific cancer studies that utilise the thawed samples optimally.
- To implement and improve infrastructural data systems for tracking information on the biological samples, including serum volumes and the generation of retrieval and shipment lists in research projects.
- To exploit the possibilities of Registry linkages to obtain additional relevant information on the donors in Janus for cancer research.
- To utilise modern technology to enable the use of the Janus biobank in gene-environment-interaction studies.
- To examine further the utilisation of the samples for research on non-cancer endpoints.

11 Evaluation of Janus as a resource in cancer research -international perspectives

Joakim Dillner

International biobank collaboration is useful both for making biobank-based studies more informative as well as for furthering the progress in the scientific field of the biobanking itself. Basically, the international biobank-based study aims to ameliorate some of the major problems encountered in modern molecular epidemiology, most notably i) unreliable study designs ii) insufficient statistical power iii) confounding, "part of the truth" study designs and non-generalisability iv) multiple hypothesis testing v) insufficient quality control, feasibility assessment and prioritisation of efforts from the perspective of several different disciplines. As the build-up and use of biobanks is expanding rapidly with many new uses and increasing demands on the infrastructure, there is a need for the scientific personnel of the biobanks to interact in scientific networks aimed to promote exchange of experiences and further the science of biobanking. Several major international biobank collaborations exist and very ambitious initiatives have been made in recent years. The Janus biobank has unquestionably pioneered the science of biobanking, particularly regarding purpose-oriented quality of biobanking and is likely to continue to be an important contributor to international research on biobanking and its uses in cancer research.

When reliable information from registries and questionnaires, in particular concerning cancer incidence and mortality, family history, health and life-style factors, can be linked to biological samples from large numbers of individuals, an enormous study base for innovative cancer research is created. The significant cost and limited throughput for obtaining data from biospecimens used to favour epidemiological studies based only on registry analyses. However. the technological advances in the analysis of biospecimens have greatly improved our ability to investigate the genetic and environmental determinants of cancer and response to treatment of cancer. Today, the bottleneck for the progress is not the analysis cost or capacity, but rather the availability of large and well characterised sample collections that have been followed-up for significant amounts of time.

Biobank-based sciences of today are however faced with a number of unresolved problems, regarding fundamental issues like integrity, ownership/custodianship and quality. The mode of operation of the Janus biobank is in many respects a "role model" for high quality biobanking efforts, where solutions to many of the current problems were, with incredible foresight, established decades ago.

In terms of ownership/custodianship, much of the current debate has focused either on "Big Brother"-issues related to governmental biobanking initiatives or on issues of making profit from personal samples/data related to commercial biobanking. The Janus biobank is a role model of a citizen biobank established from a popular grass roots movement and from the start had a clear ownership organisation (the Norwegian Cancer Society) with widespread popular trust.

In terms of protection of integrity, the Janus biobank has again acted as a role model as the personal identifiers of donors are never released to the scientists requesting withdrawal of samples. With the absence of any instance of violation of integrity after >35 years of operation in large-scale biobanking research, it can be considered as shown that biobanks can be established and used for cancer research without violation of personal integrity, provided that equally strict modes of operation as used by the Janus biobank are implemented.

In terms of quality, the principles used by the Janus Steering Board are gradually being recognised as a preferable mode of operation of biobanks. In brief, the use of the Janus biobank is decided by the multidisciplinary committee, that evaluates proposals for withdrawals of data and samples with respect to the common pitfalls that impair quality and usefulness of samples (biased study designs, insufficient power, confounding ("part of the truth" study designs that investigate only a single risk factor), multiple hypothesis testing, insufficient quality control, feasibility assessment and prioritisation of efforts from the perspective of several different disciplines). The Janus Steering Board has stringent policies for evaluation of these aspects of proposal. Prioritisation is made with the ex-

plicit aim to ensure that future research possibilities are not impaired, which includes e.g. stringent assessment to ensure minimal consumption of samples and active requests for applicants wishing to do similar studies to collaborate. The hypotheses to be tested must be filed with the Janus Steering Board in advance of approval, guarding against uncontrolled multiple hypothesis testing and duplication of efforts.

An important quality assurance is the policy that the samples must always be analysed, with the analysing laboratory being blinded to the identity of samples. The code with data on case-control status and accessory data is only released after a copy of the laboratory measurements have been filed with the Janus biobank (please observe that the code for personal identities is never released, only the clinical information needed for the statistical analyses). This policy is essential for several reasons. The strict blinding policy is an effective measure for prevention of fraud, thereby ensuring credibility and resulting impact of studies that emanate from the Janus biobank. Keeping the code until data is returned is also an effective policy to ensure control of what the biobank is being used for, which is the essence of quality. Quality is defined as being appropriate for the intended purpose and any quality improvement programme must start with assessing whether the uses and the results from the uses are in accordance with the purpose of the biobank. The present special issue should further contribute to promoting excellence in the science of biobanking, as widespread recognition of the availability of the resource will assist in ensuring that the Janus biobank is optimally used for its designated purpose – contributing to the progress in cancer research.

Several collaborative studies and international biobanking networks are currently actively attempting to develop uniform methods and quality standards for biobanking - so-called Good Biobanking Practice. All such efforts recognise the importance of establishing quality criteria concerning the nature of the sample, conditions of sample storage, the adequacy of available information et cetera, and the stringent Janus biobank policies that enable assessing quality of use in relation to purpose are gradually gaining acceptance as an essential part of Good Biobanking Practice.

That these policies are successful is evident, as the scientific output from the Janus biobank is not only voluminous but contains a substantial proportion of high impact papers that have made a decisive difference for our opportunities for cancer control.

An important aspect of the operation of the Janus biobank, is an active pursuit of research on biobanking. In the past few years, there has been a growing awareness that biobanking is not merely a service but that the complexity and innovation involved clearly merit designating biobanking as a science in itself. The doctoral thesis of Randi Gislefoss on long-term stability of serum components in biobanked specimens is a prominent example of the importance given to an evidence-based approach to biobanking.

The integration of the Janus biobank with the Cancer Registry of Norway is an internationally unique arrangement that also should be recognised as a role model. Many biobank cohorts are based on small and selected groups of volunteers. However, it is an essential feature of epidemiological cancer research that it should be population-based. The vision for the biobanks of the future should be as a biological part of the Cancer Registry, where the samples are either comprehensive population cohorts and/or systematically enrolled, comprehensive series of cancer patients. There should not be a disconnection between the registries with data and the registries with samples (i.e. biobanks) regarding how inclusive they are or the framework within which they work. To my knowledge, the Cancer Registry of Norway is the first Cancer Registry in the world to operate a large-scale biobank in-house. With the continuing scientific success of the Janus biobank, I am convinced that this concept will be followed by many other registries all over the world.

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